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(54) Title: GENE THERAPY FOR OBESITY

(57) Abstract

Gene therapy can treat obesity in mammals. An obesity regulating gene is delivered to a mammal. Preferably, the gene encodes letpin or a leptin receptor. The protein which is delivered and expressed *in vivo* is more effective than protein which is injected into the animal.

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TITLE OF THE INVENTION

GENE THERAPY FOR OBESITY

FIELD OF THE INVENTION

This invention relates to methods of gene therapy by vector-assisted delivery of a peptide hormone, and to transgenic non-human mammals so produced. This invention also related to gene therapy for obesity. This invention also relates to vectors useful in this gene therapy.

BACKGROUND OF THE INVENTION

There are numerous potentially therapeutic hormones which are peptides or proteins, including leptin, insulin, calcitonin, erythropoietin, (EPO), growth hormone, interferons, interleukin-2, hemophilia factors, vascular endothelial growth factors such as VEGF, granulocyte-macrophage colony stimulating factor, alpha 1 anti-trypsin, and others. Many have been purified, extensively studied, and even produced recombinantly and administered in a clinical setting. One problem with peptide and/or proteins as therapeutic agents, however, is that they cannot be made into conventional oral dosage forms, as contact with gastric juices will destroy the peptide. Instead, they have to be delivered by injection, intravenously, intranasally or other non-oral routes which are often not convenient for chronic usage, and may add to the expense of the drug therapy. In addition, protein injection is frequently of short duration of action and requires repetitive dosing.

Leptin is a protein expressed by the *ob* gene. Leptin is secreted by adipose tissue and appears to be both a satiety factor and a regulator of metabolism (Levin *et al.*, 1996 *Proc. Natl Acad. Sci. USA* 93:1726-1730). Both the mouse gene and its human homologue have recently been identified and sequenced (Zhang *et al.*, 1994 *Nature (London)* 372:425-431.)

Mice which are homozygous for the *ob* gene (*ob/ob)* are obese, perhaps due to leptin deficiency. When *ob/ob* mice are given daily injections of recombinant protein, their food intake was markedly

inhibited and they experienced a reduction in body weight and fat. In lean (i.e. wild-type) mice, daily injections of leptin lead to modest decreases of food intake and body weight. The results for body fat have been confirmatory to the effect of leptin on fat metabolism. (Pelleymounter *et al.*, 1995, *Science* 269:540-543; Halaas *et al.*, 1995 *Science* 269: 543-546; and Campfield *et al.*, 1995 *Science* 269:546-549).

Obesity in humans is a major disorder associated with mortality, and may result from a number of causes, and at least some may be due to an insufficient amount of leptin produced or resistance. Since leptin is a protein, and vulnerable to breakdown and inactivation by the gastrointestinal system, it cannot be delivered orally. It would be desirable to develop a therapy for leptin delivery for obese patients whose obesity is due, at least in part, to a paucity of leptin or resistance to the sustained peripheral levels.

BRIEF DESCRIPTION OF THE INVENTION

This invention relates to gene therapy wherein a viral vector is used to deliver a protein or peptide which is also a hormone. One aspect of this invention involves a method of treating a condition which is caused at least in part by an insufficient production of or resistance to a peptide or protein hormone in a mammal comprising administration of a viral vector comprising a gene encoding the peptide or protein hormone. It has been found, in accordance with this invention, that the amount of protein or peptide hormone needed to produce a biological result is much lower when the protein or peptide hormone is made by the mammal receiving the gene therapy, as compared to the amount needed to produce the same biological result when the peptide or protein hormone is delivered to the mammal by, e.g. muscular injection or intravenous administration. Further, the mammal which is expressing the gene which has been introduced by gene therapy does not develop antibodies to the peptide or protein hormone. In contrast, a mammal which receives the same peptide or protein hormone by injection or intravenous administration, may develop antibodies to the hormone.

Thus one aspect of this invention relates to a method of treating a condition caused, at least in part by an insufficient production of a peptide or protein hormone in a mammal comprising administration of a viral vector comprising a gene encoding the peptide or protein hormone wherein the gene encodes a hormone selected from the group consisting of: leptin, insulin, calcitonin, erythropoietin, growth hormone, interferons, interleukin-2, a hemophilia factor, a vascular endothelial growth factor, granulocyte-macrophage colony stimulating factor, and alpha 1 anti-trypsin.

This invention is also related to gene therapy for obesity. One aspect of this invention involves a method of treating obesity, lowering serum glucose levels or lowering serum insulin levels in a mammal in need of such therapy comprising delivering a gene encoding an obesity regulating gene to said mammal; and allowing sufficient time to pass for transcription and translation of the obesity regulating gene.

Some types of obesity are caused by an insufficient amount of leptin. Along with obesity the individual may also experience elevated serum glucose and/or insulin levels. Thus, another aspect of this invention is a method of treating obesity, elevated serum glucose levels, or elevated insulin levels comprising delivering a gene encoding leptin to a mammal wherein transcription and translation of the gene occurs *in vivo*. By "insufficient amount of leptin produced" it is envisioned that the mammal may produce functional leptin, but at lower levels than required; alternatively the animal may have a complete inability to produce leptin; or in yet another alternative, the animal produces a mutated form of leptin which either functions less efficiently than native leptin or does not function at all.

Non-human mammals, particularly rodents such as mice and rats which have received peptide or protein hormone transgenes and which express the peptide or protein hormone make up another aspect of this invention. Specifically, mice which have received the leptin gene transgenically and which express leptin, form another aspect of this invention. Mice may be *ob/ob*, *Ob/?* or homozygous wild-type for the

ob gene prior to receipt of the leptin gene. Progeny of these mammals make up yet another aspect of this invention.

Yet another aspect of this invention are viral vectors for the delivery of peptide or protein hormone genes to be used in gene therapy. In one embodiment adenoviral vectors are provided, including those with deletions in all viral protein coding sequences, which are less immunogenic than previous vectors.

Still further aspects of this invention include mammalian cells transformed with vectors of this invention.

Another aspect of this invention is a method of determining whether a compound has the ability to modulate leptin activity *in vivo* comprising administering the compound to a transgenic animal of this invention and monitoring the animal's reaction to the compound. In this way, leptin agonists, antagonists and mimetics may be identified, or if a compound shows leptin modulating activity in *in vitro* assays, it can be determined if this activity is retained *in vivo*.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a photograph of two *ob/ob* mice. The mouse on the right is from a control group. The mouse on the left received gene therapy in accordance with the pilot study (Example 3) of this invention.

Figure 2 is a graph showing the body weight changes of mice treated with recombinant human leptin protein as described in the Example 3 pilot study. Injections of human recombinant leptin were given daily IP, at 1 mg/gm body weight. Arrows indicate bleeding points.

Figure 3 is a graph showing body weight changes of mice of the pilot study treated with adenovirus carrying a reporter gene (β-galactosidase), used as a control.

Figure 4 is a graph showing body weight changes of mice of the pilot study treated with adenovirus carrying the human leptin gene.

Figure 5 is a graph showing the percent of body weight changes for all groups of mice of the pilot study.

Figure 6A (left graph) is a graph showing the amount of human leptin found in the plasma of mice treated with adenovirus containing the leptin gene as described in Example 3. Figure 6B (right graph) shows results for mice in the pilot study treated with five daily injections of recombinant human leptin.

Figure 7A (left graph) shows the amount of insulin and leptin in plasma of mice of the pilot study treated with adenovirus containing the leptin gene. Figure 7B (right graph) shows the amount of insulin and leptin in plasma of mice of the pilot study treated with recombinant human leptin injections.

Figure 8 shows glucose levels of the mice of the pilot study treated with either recombinant leptin, reporter gene or adenovirus containing the leptin gene.

Figure 9 compares body weight changes in the mice of the pilot study which received human leptin gene (Figure 9A), with mice of the expanded study (Example 5) which received mouse leptin gene (Figure 9B) and with mice of the expanded study which received human leptin gene (Figure 9C).

Figure 10 shows the body weight changes in *ob/ob* mice which were used as controls. Figure 10A is the pilot study mice; Figure 10B shows the expanded study mice which received iv. injections of dialysis buffer; and Figure 10C shows the expanded study mice which received the control adenoviral injection. The arrowhead shows the first day that injections occurred. All controls gained weight.

Figures 11A-C show the results of IP injections of human leptin. Figure 11A is the pilot study mice. Figure 11B shows mice in the expanded study which received control injections, and Figure 11C shows mice in the expanded study which received leptin injections.

Figure 12 is a graph summarizing percent body weight changes for all the treatment groups of the expanded study.

Figure 13 is a graph summarizing the mean body weight changes for the *ob/ob* mice in the expanded study.

Figures 14A-B summarize the levels of human leptin found in the plasma of mice in the expanded study. Figure 14A shows mice which received adenovirus carrying human leptin gene, and Figure 14B shows mice which received human leptin IP injections.

Figures 15A-B summarizes findings relating to the injection of recombinant leptin in the expanded study. Figure 15A shows serum levels of human or mouse leptin and weight for animals receiving leptin genes. Figure 15B shows leptin levels and weight for animals receiving leptin injections, and notes the antibody response.

Figure 16 is a graph illustrating glucose levels of animals in the expanded study.

Figure 17A shows glucose levels in adenovirus-treated mice in the expanded study and Figure 17B shows the same in mice receiving protein injections.

Figure 18 shows food intake in relation to weight loss for the expanded study.

Figure 19 shows body weight changes in treated lean mice relative to day 0 for the expanded study.

Figure 20 shows the food intake relative to control for mice treated in the expanded study with adenovirus containing leptin or recombinant leptin.

Figure 21A shows leptin levels and weight changes of lean mice treated in the expanded study with adenovirus carrying leptin genes. Figure 21B shows leptin levels and weight for lean mice receiving recombinant leptin IP.

Figure 22 shows glucose levels in lean (*Ob/?*) mice in the expanded study.

Figure 23 shows anti-adenovirus antibody levels and anti-leptin antibody levels measured after day 15 in the expanded study.

Figure 24 shows anti-adenovirus antibody levels and anti-leptin antibody levels measured after day 15 in the expanded study.

Figure 25A illustrates the HD-Leptin vector. The DNA composite fragments of HD-leptin inserted into pBluescript llKS are (left to right): the left end terminus of Ad5, composed of the inverted

terminal repeat sequences and the packaging signal ψ (nucleotides (nt.) 1-440, solid arrow), the PmeI-EclXI 16054 bp fragment of HPRT (nt.1799-17853 of HUMHPRTB, shaded bar), the leptin expression cassette, composed of the HCMV promoter, the murine leptin cDNA (500 bp) and the bovine growth hormone poly A tail (open bar), the HindIII 9063 bp fragment of C346 cosmid (nt. 12421-21484 of HUMDXS455A, shaded bar) and the right end terminus of Ad5, composed of the ITR sequence (nt. 35818-35935). The ITRs are flanked by unique PmeI restriction site used to liberate the vector from the plasmid backbone prior to the initial transfection into 293-cre4 cells for viral propagation and rescue.

Figure 25B illustrates detection of leptin protein expression mediated by the HD-leptin virus *in vitro* using a Western blot with polyclonal antibodies. Details are provided in Example 6.

Figures 26 A-F show effects of HD-leptin and Ad-leptin in lean mice. In each graph: solid circle is HD-leptin; circle with cross is Ad-leptin; open circle is AD-β-gal; and open triangle is dialysis buffer. Figure 26A shows serum leptin levels collected 2-3 times weekly (mean ± SEM). Figure 26B shows weight (mean ± SEM) measured daily. Figure 26C shows food intake measured daily. Figure 26D is a Northern Blot of RNA extracted from liver of Ad-leptin at 2, 4, and 8 weeks. Figure 26E is serum glucose measured in all animal groups (mean ± SEM). Figure 26F is insulin levels measured in all animal groups (mean ± SEM).

Figures 27 A-F show effects of HD-leptin and Ad-leptin in *ob/ob* mice. In each graph, solid circle is HD-leptin; circle with cross is Ad-leptin; open circle is AD-β-gal; open triangle is dialysis buffer; as asterisk is lean control values plotted for relative comparison. Figure 27A shows serum leptin levels collected 2-3 times weekly (mean ± SEM). Figure 27B shows weight (mean ± SEM) measured daily. Figure 27C shows food intake measured daily. Figure 27D is a Northern Blot of RNA extracted from liver of Ad-leptin and HD-leptin at 1, 2, 4, and 8 weeks. Figure 27E is serum glucose measured in all animal

groups (mean ± SEM). Figure 27F is insulin levels measured in all animal groups (mean ± SEM).

Figure 28 shows phenotypic correction of HD-leptin treated *ob/ob* mice. From left to right, representative *ob/ob* mouse treated with HD-leptin at day 54 post treatment next to a littermate treated with Ad-leptin 54 days post treatment. Lean control mouse and untreated *ob/ob* mouse are provided for comparison.

As used throughout the specification and claims, the following definitions apply:

"Native" a gene or protein is native if it naturally occurs in a given organism.

"Transgene": a gene which has been introduced into a mammal or its ancestor using a viral vector.

"Transgene construct" or "expression cassette" means a transgene and associated DNA, such as promoter(s) enhancer(s), and terminal sequences needed for control of transcription and translation of the transgene.

"Leptin gene": a gene from any mammal which encodes a native leptin, or a derivative thereof. A "derivative" is a modified leptin molecule which retains at least 80% of the biological activity of native leptin.

"Protein or peptide hormone" any hormone which is in the form of a protein or peptide, whether or not post-translationally modified. Examples include: leptin, insulin, calcitonin, EPO, growth hormone, interferon, IL-2, vascular endothelial growth factors such as VEGF, GMCSF and alpha 1 anti-trypsin.

"Obesity regulating gene": a gene whose gene product is involved in the regulation of obesity in a mammal, including genes encoding leptin, leptin receptors, neuropeptide Y, and the like.

"*ob/ob*" means the animal is deficient in leptin.

"lean" means the animal expresses normal levels of leptin and genotypically are homozygous normal for the leptin gene.

"*Ob/?*" means the animal expresses leptin, but it is unknown if the animal is homozygous normal or a carrier of the leptin gene defect.

"bp" means base pair

"IV" or "iv" means intravenous injection.

"IP" means intraperitoneal injection.

"IM" means intramuscular injection.

"Ad" means adenovirus.

"Ad-leptin" means an adenovirus vector carrying a leptin transgene.

"HD" means helper-dependent virus system.

"HD-leptin" means a helper dependent virus carrying a leptin transgene.

"wild-type" or "wt" refers to the non-mutated form of a gene. With reference to the *ob* gene, a mouse with a wild-type phenotype is lean.

In the past, recombinant peptide hormones have been administered to mammals (including man) suffering from conditions caused at least in part by an insufficiency of the hormone. Examples include replacement of congenital (e.g. hemophilias) or acquired (e.g. erythropoetin) deficiencies. Enhanced expression of loci that might provide a therapeutic benefit (e.g. LDL receptor or apolipoprotein A-I) may best be accomplished by very long-term or permanent expression with tissue-specific and physiologic regulation. These conditions include diabetes, alpha 1-anti-trypsin deficiency, etc. However, one problem with such therapy is the inconvenience and associated expense of an injection or iv administration. Also, the recipient may develop antibodies to the administered hormone.

Recombinant leptin has been administered to animals who exhibiting an obese phenotype, and a daily injection has been shown to decrease body weight. There are numerous disadvantages to this method of treating obesity, however. Injections are not a particularly convenient method of treatment, particularly for long-term treatments. In addition, the half-life of leptin is short, so the duration of a single

treatment was found to be only about 24 hours, after which the animals were observed to re-gain weight.

It has been found, in accordance with this invention, that a peptide or protein hormone which is expressed *in vivo* is more advantageous than administration of the recombinant form of that hormone; its effects last longer, and most surprisingly, is up to 20 fold more potent than recombinant peptide hormone administered by injection. Further, if the recipient mammal endogenously produces the hormone, no immune response with respect to the transgenic hormone is observed.

This invention utilizes the leptin gene delivered to *ob/ob* mice as a model for peptide hormones in general. Leptin was chosen because its nucleic acid and amino acid sequences were known, and its effects on obese *ob/ob* mice are visually apparent as well as biochemically apparent. Applicants are not aware of any reason why their findings for leptin are not generally applicable to all peptide or protein hormones, and intend for this invention to be construed broadly.

Gene Constructs

The sequences of leptin and leptin genes from various species are known (Zhang *et al.*, 1994 *Nature* 372:425; Ogawa *et al.*, 1995 *J. Clin. Invest.* 96:1647-1652; Murakami *et al.*, 1995 *Biochem. Biophys. Res. Commun.* 209:944; and Considine *et al.*, 1995 *J. Clin. Invest.* 95:2986; each of which is hereby incorporated by reference). If desired, genes encoding leptin derivatives may also be used. Since the amino acid and nucleotide sequence of leptin is known, it is well within the skill of one of the ordinary artisan to construct a nucleotide sequence which encodes a desired mutant form of leptin. These can be used to study structure and function relationships involved in leptin binding and signaling in the transgenic animal model.

The gene which encodes the leptin should also contain at least one element which allows for expression of the gene when introduced into the host cell environment. These sequences include, but are not limited to promoters, response elements, and enhancer elements.

In a preferred aspect of this invention, promoters are chosen which are regulatable; i.e. are inducible rather than constitutive. Particular examples of such promoters include: the Gene Switch™ mifepristone inducible gene regulation system commercially available from Gene Medicine; the "two component gene regulation system" commercially available from Ariad, regulatable tet, P-450, and constitutive promoters such as EF-1 alpha, SR-alpha, CMV, albumin and the like.

Vector

The heterologous leptin gene may be delivered to the organism using a vector or other delivery vehicle. DNA delivery vehicles can include viral vectors such as adenoviruses, adeno-associated viruses, helper dependent adenoviruses, and retroviral vectors. See, for example: Chu *et al.*, 1994 *Gene Ther.* 1:292-299; Couture *et al.*, 1994 *Hum. Gene Ther.* 5:667-277; and Eiverhand *et al.*, 1995 *Gene Ther.* 2:336-343. Non-viral vectors which are also suitable include DNA-lipid complexes, for example liposome-mediated or ligand/poly-L-Lysine conjugates, such as asialoglyco-protein-mediated delivery systems. See for example: Felgner *et al.*, 1994 *J. Biol. Chem.* 269:2550-2561; Derossi *et al.*, 1995, *Restor. Neurol. Neuros.* 8:7-10; and Abcallah *et al.*, 1995 *Biol. Cell* 85:1-7.

If a viral vector is chosen as the delivery vehicle it may be one which is capable of integrating into the host genome, so that the gene can be expressed permanently, but this is not required. In cases where the vector does not integrate into the host genome, the expression of the gene may be transient rather than permanent.

One vector which is suitable for transient expression of the *ob* gene is an adenovirus which has a deletion in the E1 gene. Such vectors are known, as taught in the aforementioned WO 95/00655 and Mitani *et al.*, 1995 publications. These viruses preferentially infect hepatocytes, where they persist for approximately 3-4 weeks after the initial infection. While in the hepatocytes, these viruses can express the heterologous gene.

The vector is administered to the host, generally by iv injection but may be intramuscular, intraperitoneal, oral, subcutaneous or other form of delivery. Suitable titers will depend on a number of factors, such as the particular vector chosen, the host, strength of promoter used and the severity of the disease being treated. For mice, an adenovirus vector is preferably administered as an injection at a dose range of from about 5.0 x 10^6 to about 10 x 10^6 plaque forming units (PFU) per gram body weight. Preferred dosages range from at least about 6-9 x 10^6 PFU/gm body weight, and more preferred is from at least about 6.7-8.6 x 10^6 PFU/gm body weight (equivalent to approximately at least 1x 10^7 to 1-5 x 10^8 PFU for mice). Higher amounts are also useful, and up to 10^{12} particles may be used.

If the effect desired is a permanent one rather than a transient one, it is preferred that a helper dependent viral vector be utilized.

Adenoviral (Ad) vectors are currently among the most efficient gene transfer vehicles for both *in vitro* and *in vivo* delivery, but the utilization of first generation Ad for many gene therapy applications is limited due to the transient nature of transgene expression obtained by these vectors. Several factors have been shown to contribute to and modulate the duration of Ad-mediated gene expression as well as the immunogenicity of these vectors, including "leaky" viral protein expression and the transgene delivered. The development of Ad vectors, deleted in all viral protein coding sequences, offers the prospects of a potentially safer, less immunogenic vector with an insert capacity of up to approximately 37 kb. This vector requires supplementation of viral regulatory and structural proteins in trans for packaging and rescue, thus helper dependent (HD).

The development of Ad vectors, deleted in all viral protein coding sequences, has resulted in a less immunogenic vector with an insert capacity of up to approximately 37 kb. This class of vectors requires supplementation of viral regulatory and structural proteins in *trans* for packaging and rescue, and are thus termed "Helper Dependent" (HD). These are further described in Parks *et al.*, 1996 *Proc. Natl.*

Acad Sci. 93:13565-13570, and U.S. Patent Application Serial No. 08/488,014, filed June 7, 1995 both of which are hereby incorporated by reference.

In a preferred embodiment, the HD vector containing a leptin transgene construct (HD-leptin) comprises the Ad5 inverted terminal repeats (ITR) and packaging signal sequences, a leptin transgene construct, and "stuffer" DNA. Stuffer DNA is human genomic DNA sequences or other non-transcribed DNA sequences used to increase the vector insert size to at least approximately 28 kb. In one embodiment of this invention, the stuffer DNA is a segment of the human hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene. In a particular embodiment of this invention, pSTK120, the stuffer DNA is intronic HPRT which also contains a matrix association region (MAR). The MAR has been shown to confer DNA attachment to the nuclear matrix. A genome of this size is preferred, as the virus is more stable and productive. An HD-leptin vector is illustrated in Figure 25A.

A specific embodiment of this invention is vector pSTK120 comprises a first segment of adenovirus a first segment of stuffer DNA, a transgene DNA, construct a second segment of stuffer DNA, and a second segment of adenovirus DNA. The adenovirus DNA is preferably adenovirus type 5 DNA sequences, and together comprise adenovirus inverted terminal repeats (ITRs) that comprise the viral origin of replication and packaging signals. The non-viral DNA can contain up to 38 kb. An important aspect of the vector is that no adenoviral proteins are expressed in the host cell. The first segment of adenovirus type 5 DNA preferably comprises at least about nucleotides 1-440, although more may be present. The second segment of adenovirus type 5 DNA preferably comprises at least about nucleotides 35818-35935.

For propagation of HD-leptin, a helper virus system containing a modified E1 deleted vector with *lox* sites flanking the packaging signals (Ad LC8cluc) and a 293 cell line expressing *Cre* recombinase such as 293-*Cre4* is preferred. Such systems are generally known and are described in: Parks *et al.*, 1996, *supra*; and Edwards

et al., 1990 *Genomics* 6:593-608, and U.S. Patent Applications 08/250,885 (filed May 31, 1994); 08/473,168 (filed June 24, 1995); and 08/719,217 each of which is hereby incorporated by reference.

Hosts

Animals which transiently or permanently express a peptide or protein hormone such as the *ob* gene product are valuable research tools. For example, they can be used to monitor the effects of decreasing amounts of leptin, or the effect of various exogenously supplied substances (such as hormones or putative leptin receptor agonists and antagonists) in an environment of decreasing leptin availability.

In addition to making animal models useful in studying various aspects of obesity, this invention is specifically directed to gene therapy for humans.

In accordance with this invention, an adenovirus or helper dependent adenovirus containing a transgenic leptin gene (human or murine) is administered to mice which are obese (*ob/ob*). Although the leptin gene (or a derivative) from any desired species may be used, in preferred embodiments, the gene which is from the same species as the host is used. These were compared with *ob/ob* mice which have received adenovirus containing only a marker gene (β-galactosidase), those which did not receive gene therapy but which did receive injections of recombinant leptin, and to untreated controls. Further controls used in some of the experiments are *db/db* mice (obese, but unresponsive to leptin injections due to a receptor defecit), and lean mice (wild-type phenotype, genotype *Ob/ob* or *Ob/Ob*).

RESULTS OF THE PILOT STUDY (Example 3)

Body-weight: Figure 2 illustrates the body weight changes for the *ob/ob* mice of Example 3 ("pilot study") receiving 1 mg/gm body weight human recombinant leptin protein injections daily, compared to untreated controls. Animals receiving leptin were injected for five consecutive days, shown by the darkened symbols on the graph.

All the animals receiving the leptin lost weight within 24 hours post-injection. All animals gained weight within 48 hours after the last IP injection. Figure 3 illustrates the weight measured for these mice receiving various titers of an adenovirus carrying the reporter gene. All animals continued to gain weight post injection. Figure 4 shows the results for the mice receiving an adenovirus carrying the leptin gene. As can be seen from the graph, all animals lost weight within 24 hours post injection. They continued to loose weight for 1-2 weeks post treatment. The injections were effective over a relatively large titer range, and a dose-effect was noted.

Along with absolute changes in weight, the percentage body weight change was calculated for all groups in the pilot study. In the animals treated with recombinant leptin injections, weight loss plateaued at day three, and from day 1-5 post treatment, a 4.7% loss in body weight was noted. For mice treated with the vector carrying the leptin gene, weight loss persisted over a 10-12 day period, and resulted in an 18.61% loss in body weight. Furthermore, over days 1-5 post treatment, a 9.17% loss in body weight was observed compared to only 4.7% loss in the recombinant leptin treated mice. This is illustrated in Figure 5.

Leptin: The amount of human leptin in plasma was measured in the pilot study animals which received injections of human recombinant leptin and those which received the vector carrying the leptin gene. Those receiving the recombinant protein were noted to have leptin levels which were approximately 20-fold higher than the amount of leptin found in control (lean, wild type) animals; peak amounts of 399.8 ± 40.91 ng/ml. Those receiving the leptin gene had levels of leptin in their plasma which was within the normal range found in a wild-type mouse (17.52 ± 4.66 ng/ml). In both groups of animals of the pilot study, weight gain was synchronized with the fall of human leptin detected in the plasma. This is illustrated in Figure 6.

Insulin: The amount of insulin in the plasma was measured in the pilot study animals receiving recombinant protein and those which received the gene therapy. This is illustrated in Figure 7. In both

groups, insulin levels were observed to drop to those found in lean (wild-type) levels and was inversely correlated to leptin levels. In the mice receiving gene therapy, the low insulin levels were sustained for at least one week whereas in the recombinant leptin-treated mice, insulin levels increased to pre-treatment levels within 24 hours post injection.

Glucose: The levels of glucose in plasma was also measured in pilot study mice receiving recombinant leptin and those receiving the gene therapy treatment. In both of these groups, the glucose levels dropped within 6-9 days post treatment. The recombinant protein-treated mice did not achieve levels comparable to those found in lean, wild-type mice, and only sustained the lower level for less than one week. On the other hand, the mice which received the gene therapy had reductions in glucose levels to that of wild type lean mice, and they sustained this reduced level for at least two weeks. This is illustrated in Figure 8.

RESULTS OF THE EXPANDED STUDY

An expanded study was also conducted, and is detailed in Example 5. This differed from the pilot study in that somewhat older animals were used, additional investigations were performed, and wild-type lean mice were also treated. The observations are set forth below.

Weight loss: The *ob/ob* mice which were treated with adenovirus carrying the human leptin gene lost weight, (18.61% over a 10-12 day period) as did the mice in the pilot study, as is shown in Figure 9. Weight loss occurred within 24 hours, with 9.17% loss from day 1-5 post treatment. Weight gain was restored within 10-20 days post-treatment. On the other hand, mice receiving control treatments continued to gain weight throughout the experiment, as did their counterparts in the pilot study, as shown in Figure 10.

The *ob/ob* mice receiving iv injections of adenovirus carrying the mouse leptin gene lost more weight than did mice receiving iv injections of adenovirus carrying the human gene. The pattern of weight loss and time of weight gain were substantially the same as those

receiving the human gene, however. This is shown in Figures 9B and 9C.

Mice receiving IP injections of recombinant leptin protein lost weight within 24 hours, and plateaued at day 3 of the daily injection, as illustrated in Figure 11. After day 1-5 post-treatment, loss was 4.7%, significantly less than that of groups receiving gene therapy. Control animals gained weight throughout the experiment, as can be seen in Figures 11B and 11C.

A summary of the percent of body weight changes is given in Figure 12. The noteworthy points are as follows:

- *ob/ob* mice which were treated with the reporter vector, and *db/db* mice which were treated with β-gal, the *ob* gene vector or recombinant protein all continued to gain weight post-treatment.
- *ob/ob* animals which were treated with adenovirus carrying human leptin gene or which were treated with recombinant leptin lost weight within 24 hours post injection.
- In *ob/ob* mice treated with adenovirus containing a human leptin gene, the observed weight loss persisted over a 10-12 day period and resulted in an 18.61% loss in body weight. In day 1-5 post treatment, there was a 9.17% loss in body weight.
- In *ob/ob* mice treated with recombinant leptin, weight loss plateaued at day 3 of the daily injection (1 mg/gm). In day 1-5 post treatment a 4.7% loss in body weight was observed.
- Weight gain observed in the mice treated with the human leptin gene resumed after 10-12 days post treatment at a rate identical to that observed pre-treatment.

The summary of the mean changes in body weight of *ob/ob* mice are presented in Figure 13. Table 1, below presents the percent weight change as calculated over various time periods post treatment.

TABLE 1

% Weight Change Post Treatment	Days 1-5	Day 8	Day 12
iv. leptin gene	-10.27	-16.52	-22.2
iv. control	+3.25	+4.46	+7.1
IP leptin protein	-6.46	-8.58	-11.62
IP control	+3.41	+4.33	+5.9

It was also found that there was a greater response when the adenovirus carried the mouse leptin gene than when the human leptin gene. Both of these treatments had a greater response that the IP injection of leptin protein.

Administration of IP leptin led to a response similar to that seen in the pilot study. A sharp drop in weight in the first three days was observed which was substantially identical to that observed for the gene-treated animals. This was followed by a moderation in weight reduction in the IP protein treated animals, and a bifurcation in weight reduction slopes for both treatments.

As was seen in the pilot study, the adenoviral-mediated effect was transient, and weight gain was observed at day 11-12 post treatment for the iv. injected animals and day 8 post treatment for the IM injected animals. While not wishing to be bound by theory, it appears that this is due to an immune response to the adenovirus and or adenoviral genes, and not due to an immune response to the leptin produced.

Leptin levels: Levels of leptin in blood plasma were studied in each of the mice groups, and is illustrated in Figure 14. Those which were treated with the adenovirus carrying the human leptin gene (Figure 14A) had levels which were within the normal range found in wild-type mice (averaging 12.5 ng/ml). In the group treated with recombinant protein (Figure 14B), levels of human leptin exceeded wild-type by about 20-fold; for mouse leptin, concentration exceeded

wild type by about 10-fold (Figure 15A). Weight gain was observed to be synchronized with the fall of leptin levels in plasma.

Recombinant Leptin injections: In the expanded study, leptin IP injections were continued daily over a longer time. Results were similar to those in the pilot study, however, and a summarized in Figure 15. The weight loss associated with the adenovirus treatment at any point prior to day 10-12 was about twofold greater than that observed with the leptin IP treatment. Peak plasma concentration of leptin was 20- and 10-fold higher in leptin IP treated groups than in the groups treated with adenovirus carrying the human and mouse genes, respectively. The uninterrupted daily protein injections continued to be associated with weight loss to at least day 24.

Glucose levels: The glucose levels dropped within 6-9 days post-treatment in mice receiving adenovirus carrying the leptin gene and receiving recombinant injected leptin. (See Figures 16, 17A and 17B). No change in glucose levels were observed in any control treated mice. The only treated group whose glucose levels reached that of normal (lean) mice were the mice receiving adenovirus carrying the human leptin gene, and the normal glucose levels was sustained for at least two weeks. Mice receiving recombinant leptin injections sustained reduced glucose levels for less than one week.

Food intake: An attempt was made to measure food intake, and is shown in Figure 18. Accurate measurements in the group receiving adenovirus carrying a leptin gene and in the group receiving human recombinant leptin was not possible after about one week, as the animals became hyperactive and spilled food out of their containers. In the group receiving adenovirus carrying the human leptin gene, food suppression was 76.6 % $\pm$ 6.75, whereas in the group receiving recombinant human leptin injections, suppression was 43.8 % $\pm$ 4.8.

Comparisons with lean mice: Various treatments were tried on lean, "normal" mice whose genotype was (*Ob/?*). The changes in body weight are shown in Figure 19. Those receiving adenovirus with the mouse leptin gene had a greater loss than those receiving the human gene, and this was a greater loss than those receiving the

recombinant leptin protein IP. The control mice, those receiving IM injections and plasmid vectors showed no response.

In the lean mice receiving IP leptin treatment, a sharp drop in weight was observed during the first three days, which was substantially identical to that observed in the *ob/ob* mice receiving the same treatment. In the lean mice, however, it was followed by a moderation in weight reductions, and then a plateau.

In general, the gene therapy treatment response of the lean mice resembled that of the *ob/ob* mice in that the effect was transient. The lean mice the transient duration of treatment was shorter than that of the *ob/ob* mice (6 days for lean; 10-12 days for *ob/ob*).

Table 2 presents the percent weight change in lean mice:

TABLE 2

Treatment	Baseline	Day 1-5	Day 6	Day 23
Adenovirus (IV)	22.1 ±1.2	-8.76	-10.78	-1.19
Control (IV)	21.6 ±2.1	-2.71	-2.34	+0.14
Protein (IP)	21.7 ±1.7	-5.45	-5.22	-4.66
Control (IP)	21.6 ±1.9	-1.29	-0.46	-3.42

Food intake for treated mice relative to controls was measured and is presented in Figure 20. In adenovirus-treated mice, food intake was suppressed by about 50%. In recombinant protein treated mice, food intake was suppressed by about 30%.

Leptin levels were also measured in the treated lean mice and are shown in Figures 21A (for mice receiving leptin gene) and 21B (for mice receiving recombinant leptin IP). Glucose levels in lean mice undergoing the treatments were measured and are shown in Figure 22.

Anti-leptin antibody levels and anti-adenovirus antibody levels were measured and are shown in Figures 23 and 24. Based on these responses, it is concluded that the transient expression of the leptin genes was due to an immune response to the viral proteins which were produced by the viral vector. Use of a vector which does not allow any viral proteins to be produced would result in no antibodies being

produced, and a permanent, rather than transient expression of the leptin genes.

HELPER-DEPENDENT VIRAL CONSTRUCT STUDIES

In this next study (Example 6) animals received the helper dependent virus expressing mouse leptin (HD-leptin).

Mice were treated with a single tail vein administration of $1\text{-}2 \times 10^{11}$ particles of either HD-leptin, adenovirus carrying the leptin gene (Ad-leptin) or control virus.

In the lean mice, treatment with Ad-leptin resulted in a transient increase in serum leptin levels and weight loss for only 7-10 days (Figs. 26A and 26B). In contrast, treatment with HD-leptin resulted in persistent high serum leptin levels (6-10 fold over background) and approximately a 20% weight loss for at least 2 months. Weight loss in HD-leptin treated mice was associated with satiety that persisted over a longer period (2-3 weeks) as opposed to those treated with Ad-leptin (5-7 days) (Fig. 26C). These effects can be correlated with the duration of gene expression obtained with these two vector types. Gene expression mediated by Ad-leptin was found to be very transient by northern blot analysis of total liver RNA, whereas that mediated by HD-leptin persisted for at least 8 weeks (Fig. 26D). No changes in serum glucose or insulin levels were detected through out the study in the treated lean mice (Figs. 26E and 26F).

The HD-leptin was also found to be more effective in obese (*ob/ob*) mice than the Ad-leptin vector used in the previous-described pilot and extended studies. In the *ob/ob* mice treated with Ad-leptin, serum levels of leptin increased only transiently during the first 1-4 days of treatment after which the levels declined and returned to baseline within 10 days post injection (Fig. 27A). These increased leptin levels resulted in transient body weight loss of only approximately 25% and mice began gaining weight within 2 weeks of treatment (Fig. 27B). In contrast, in the *ob/ob* HD-leptin treated mice, increasing serum leptin levels were observed up to approximately 15 days post-treatment, after which the levels gradually dropped to baseline over the subsequent 25

days. The initial rise in leptin levels correlated with rapid weight reduction resulting in greater than 60% weight loss (reaching normal lean weight) within one month. Weight loss was maintained for a period of 6-7 weeks post treatment. As leptin levels dropped to baseline a gradual increase in body weight was observed. Satiety was observed in association with increased leptin levels, and parallel to other findings food suppression was sustained for a longer period (approximately 1 month) compared to the short transient effect induced by Ad-leptin (approximately 10 days) (Fig. 27C).

Leptin specific antibodies were detected in sera of Ad-leptin and HD-leptin *ob/ob* treated mice, and it was essential to determine whether the drop observed in serum leptin levels was due to interference of the antibodies with the ELISA assay utilized to measure leptin or if the drop was due to loss of gene expression. Leptin gene expression was examined by total RNA northern blot analysis, in Ad-leptin *ob/ob* treated mice expression was transient and RNA levels were beyond the sensitivity level of detection at 1 week post treatment, where as in HD-leptin treated mice gene expression was detected up to 4 weeks post- injection but was undetectable at 8 weeks (Fig. 27C).

Serum glucose and insulin levels dropped during the first 1-2 weeks post-treatment to normal lean values in both HD-leptin and Ad-leptin treated mice, and similar to all other findings effects of HD-leptin treatment were sustained for longer periods (Figs. 27E and 27F. The subsequent increase in glucose and insulin levels in both vector treatments correlated with the drop observed in serum leptin levels. The overall HD-leptin mediated prolonged effect was also reflected in the accompanying phenotypic correction which was sustained for a duration that exceeded what was observed in litter mates treated with Ad-leptin (6-7 versus 2-3 weeks).

Preliminary analysis of liver histopathology in HD-leptin and Ad-leptin treated mice revealed differences in the extent and duration of cellular infiltrate. Moderate lymphoid inflammation was present in mice one week after injection with HD-leptin in contrast to moderate, acute inflammation characterized mainly by the presence of

neutrophils in the Ad-leptin treated mice. At two weeks, hepatic inflammation in HD-leptin treated mice was essentially indistinguishable from controls; whereas, in the Ad-leptin treated mice the immune response was not resolved and moderate lymphoid inflammation was seen. The initial inflammatory response in the HD-leptin treated mice may be a consequence of the contaminating (< 1:100) AdLC8cluc helper virus used in the production of HD-leptin vector. The resolution of the initial inflammation observed in the HD-leptin treated mice was associated with an initial drop in leptin levels followed by a stable persistent level of expressed leptin in lean mice and a prolonged period of continued gene expression in the *ob/ob* treated mice. In this study all adeno-related treatments (HD-leptin, Ad-leptin and Ad-β-gal) were associated with the generation of neutralizing antibodies, and secondary intravenous administrations were ineffective.

This study clearly illustrates that HD vectors achieved longer gene expression than what was observed by the first generation Ad-vector. The differences observed between the effects of HD-leptin and Ad-leptin on treated mice is directly related to the elimination of the Ad protein coding DNA sequences since the expression cassette containing the promoter (HCMV) and transgene (leptin) used in both vectors was identical. It has been shown that transgene immunogenicity plays a central role in loss of gene expression, which may explain the transient effect of HD-leptin seen in the *ob/ob* and not in the lean mice.

The leptin model used in these studies provided an excellent tool and was invaluable with regards to the specificity and sensitivity of the numerous parameters used to indirectly and directly follow relative changes in gene expression. While not wishing to be bound by theory, the differences between the longevity of expression mediated by the HD deleted vector and the transient effect observed by others, may reflect differences in the size of the recombinant virus used as it pertains to its stability and efficient packaging which have been characterized. Further, stuffer DNA may play a role in improving stability of gene expression. The HD-vector system of this invention thus reflects a significant advance over previous Ad vectors with regards to vector

capacity and reduced immunogenicity in relation to viral protein expression, they have and thus wide application in gene therapy.

Thus another aspect of this invention is a method of permanently expressing a transgenic peptide or protein hormone gene *in vivo* by administering the gene to a mammal using a helper dependent adenoviral vector, wherein the mammal also endogenously expresses a non-transgenic version of the peptide or protein hormone gene. While not wishing to be bound by theory, it appears that the results for the *ob/ob* mice and the wild-type mice can be explained as follows. In the *ob/ob* mice, antibodies against leptin were observed, whereas in wild-type, no anti-leptin antibodies were formed. Thus in animals whose immunological system has experience with leptin, the transgenic leptin and endogenously produced leptin were immunologically indistinguishable. However in animals which do not endogenously produce leptin, the immunological system treats transgenic leptin as if it were a foreign protein, and mounts an antibody attack.

These findings, however have positive implications for the use of leptin (or other hormones) in gene therapy for humans. Human obesity is generally not the result of a double recessive mutation in which no leptin is produced. However in some obese patients, the amount of leptin produced is abnormally low. For these patients gene therapy with a helper dependent adenovirus carrying a leptin gene would provide a way of permanently correcting the leptin level.

Likewise then, the helper-dependent adenovirus system can be use to permanently deliver other protein or peptide hormone genes to a human patient, provided that patient endogenously expresses that hormone (even at a low level). This gene therapy can be used to raise protein or peptide hormone levels from below normal to a normal range. Examples include: insulin, calcitonin, erythropoietin, growth hormone, interferons, interleukin 2, hemophilia factors, VEGF, GMCSF, and alpha 1 anti-trypsin.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Cloning and expression of leptin

Two PCR cDNA amplification fragments were obtained from Jefferson University (generated by cloning both variants from a Clontech phage human hypothalamic library): one coding for the human leptin and one for the human leptin variant with glutamine, (Zhang *et al.*, 1994, *Nature* 372:425; Considine *et al.*, 1995 *J. Clin. Invest.* 95:2986). Both PCR fragments were amplified for cloning purposes. Two primers were designed and ordered from GIBCO BRL Custom Primers: Forward primer: ATG CAT TGG GGA ACC CTG TG (SEQ.ID.NO:1)

Reverse primer: TCA GCA CCC AGG GCT GAG GT (SEQ.ID.NO:2)

The primers were used to re-amplify the cDNA as follows: 2 µl each primer (0.3 µg/µl stock), 2 µl dNTP (10 µM, Pharmacia), 10 µl 10 X PCR Buffer (Buffer 2 from Expand Long Template PCR System Kit, Boehringer Mannheim), 2 µl Taq polymerase (Perkin Elmer), 3 µl template DNA and 18 µl water. PCR cycling conditions were as follows: Mixture was incubated at 94°C initially (without the addition of the Taq enzyme) for 1-2 minute, Taq was then added to each tube and the cycling program was initiated, 20 cycles of 94°C for 30 seconds, 45°C for 45 seconds and 72°C for 1 minute. At the end of the 20 rounds of amplification the samples were incubated at 72°C for 7 minutes. The expected fragment size in each case (human leptin - hOb, and human leptin with glutamine-hObGLN) was 501 and 504, respectively. The PCR amplified fragments were cloned into pCR-Script SK(+) plasmid (Stratagene) and several selected bacterial colonies were grown, plasmids extracted and sequenced to verify correct sequence of both cloned.

Inserts were then used for generating recombinant adenoviral shuttle vectors. The adenoviral vectors used in this study are essentially the same as those described in Morsey *et al.*, 1993 J. *Clin. Invest.* 92: 1580-86, which is hereby incorporated by reference, except

for the leptin gene insert. The pdelE1sp1CMV-BGHpA adenoviral shuttle vector, obtained from Baylor College of Medicine was used for the cloning of the two inserts (hOb and hObGLN) Similarly mOb cDNA (Zhang *et al.*, 1994 *Nature* 372:425) was inserted into pdelE1sp1CMV-BGHpA. All three shuttles (pdelE1sp1CMV-mOb-BGHpA, pdelE1sp1CMV-hOb-BGHpA and pdelE1sp1CMV-hObGLN-BGHpA) were tested for leptin expression by western blot analysis.

EXAMPLE 2

The three shuttle vectors from the previous example are used in rescue replication of the deficient E1 deleted adenoviral vectors. 293 cells, commercially available from Microbix, passage 27-30 were set up one day ahead of transfection in 60 mm dishes, and were about 70-80% confluent at the time of use.

Plates were made containing one of the shuttle plasmids and pJM17; pFG140 (purchased from Microbix Biosystems Inc.) was used as a positive control for the efficiency of transfection. Plaques were identified, and plugged out of the agarose overlay using a sterile glass Pasteur pipette. Each plugged plaque was resuspended in 100-500 µl of PBS (with calcium and magnesium) in 10% glycerol, frozen at -80°C and thawed (1-3 times). The thawed plaque was then used to infect a 90% confluent 6 cm plate of 293 cells to expand the isolated virus. 5-8 days post infection, cytopathic effects (CPE) were apparent on the cells (cells rounded up and started to detach and float in media). Cells were collected by scraping and tested for leptin expression by western analysis and for DNA restriction pattern by Hind III digestion of extracted DNA and ethiduim bromide stained gel analysis. One of the positive plaques identified based on leptin secretion and correct DNA restriction pattern was selected and used for a second plaque purification followed by a similar procedure of expansion and analysis. After the second plaque purification, the virus was propagated on a large scale. Cesium banding and titration was used to purify and quantitate.

The resulting titered viral stocks (Ad-HCMV-mOb-BGH^{PA}, Ad-HCMV-hOb-BGH^{PA} and Ad-HCMV-hOb GLN-BGH^{PA}) were stored at -80°C until use.

EXAMPLE 3

Pilot Study

Baseline determinations

Three groups of 8 week old mice were used: *ob/ob*, *db/db*, and lean (wild type, controls). All groups of mice were fed milled Purina Chow 5008 starting from day of arrival or day after arrival. Food consumption was also measured. After approximately 4 days on milled chow, the mice were weighed and bled for determination of plasma levels of glucose and insulin. Injections were started 8 days after the initiation of base line measurements but before injections, mice were weighed and blood samples were obtained from all study mice for determination of plasma glucose and insulin. Leptin level in plasma were also measured.

Mice were housed 5 per cage and fed milled Purina Chow 5008 in feed cups with lids. 24 hour food consumption was measured at the same time each day. Only after food consumption was equilibrated to a fairly constant level, usually 20-25 grams chow/5 mice-day, was virus injected.

On the day of injection but before injection, food consumption, body weight, and a baseline blood sample were taken in the morning from a snipped end of tail. Blood was collected into heparinized capillary tube (total volume approximately 70-100 µl). Hematocrit was measured, and plasma was collected.

Mice were injected as follows:

A. *ob/ob* mouse groups: iv injections, 5 mice/group

Group 1 received 2.75 x 10^8 / gm wt of AdHCMV-hob-BGH^{pA} (in 500 µl dialysis buffer) in the tail vein.

Group 2 received 2.75 x 10^8 / gm wt of AdHCMV-βgal reporter (in 500 µl dialysis buffer) in tail vein.

Group 3 received 500 ml dialysis buffer in tail vein.

Group 4 received 1 mg/kg wt active leptin daily IP injections for 5 days.

B. *db/db* mouse groups: iv injections, 5 mice/group

Group 1 received 2.75 x 10^8 / gm wt of AdHCMV-hob-BGH^{pA} (in 500 µl dialysis buffer) in the tail vein.

Group 2 received 2.75 x 10^8 / gm wt of AdHCMV-βgal reporter (in 500 µl dialysis buffer) in tail vein.

Group 3 received 1 mg/kg wt active leptin daily IP injections for 5 days.

C. Lean control mouse group: one cage of 5 mice as measure of lean parameters

No injections.

EXAMPLE 4

Leptin receptor

Adenovirus vectors are made similarly to those described in Examples 2-3, except that the leptin receptor gene replaces the leptin gene. Mice which are *db/db* are used in place of the *ob/ob* mice. Results for the *db/db* mice are similar to those observed with the *ob/ob* mice reported herein. After injection, glucose levels fall, insulin levels fall and the mice loose weight. No effect is observed in control mice and in *ob/ob* mice injected with vector carrying a leptin receptor gene.

EXAMPLE 5

Repeat and Expanded Study

Procedures followed were similar to those given in Example 3, with the following changes. Mice were older (12 weeks at the start of the experiment vs. 8 weeks) and were larger at the start of the experiment (approximately 50 grams vs. approximately 35 grams). In addition, further treatments were given, as shown below. Mice in the *ob/ob* group were treated as follows:

Group Number	n =	treatment	description of treatment
#1	10	AdHCMV-hob-BGHpA	1.5 x 10^9 pfu/mouse (in 450 µl dialysis buffer in the tail vein.
#2	10	AdHCMV-mob-BGHpA	same as #1
#3	5	dialysis buffer control	same as #1
#4	5	Ad control β-gal expression	same as #1
#5	10	hOB protein	daily (1 mg/kg initial wt in 200 µl) IP injections of active hOb for the length of the study
#6	5	vehicle control	200 µl protein suspension buffer
#7	10	AdHCMV-hob-BGHpA	IM 1.5 x 10^9 pfu (distributed equally on both quadriceps muscles, 60 µl vol).
#8	10	AdHCMV-mob-BGHpA	same as #7
#9	5	Ad control β-gal expression	same as #7

In addition, lean mice (genotype *Ob/?*) were also treated in the same way as the *ob/ob* mice above.

Lean Group Number	n =	treatment	description of treatment
#1	10	AdHCMV-hob-BGHpA	1.5 x 10^9 pfu/mouse (in 450 µl dialysis buffer in the tail vein.
#2	10	AdHCMV-mob-BGHpA	same as #1
#3	5	dialysis buffer control	same as #1
#4	5	Ad control β-gal expression	same as #1
#5	10	hOB protein	daily (1 mg/kg initial wt in 200 µl) IP injections of active hOb for the length of the study
#6	5	vehicle control	200 µl protein suspension buffer
#7	10	AdHCMV-hob-BGHpA	IM 1.5 x 10^9 pfu (distributed equally on both quadriceps muscles, 60 µl vol).
#8	10	AdHCMV-mob-BGHpA	same as #7
#9	5	Ad control β-gal expression	same as #7

Prior to treatment, the lean mice (*Ob/?* genotype) had the following baseline body weight statistics:

Treatment	n	mean	median	sd	min	max
AdHCMV-hOB (IV)	8	22.1	22.5	1.2	20.3	23.4
AdHCMV-mOb (IV)	10	21.9	21.8	1.5	19.2	24.3
Dialysis control (IV)	5	21.9	22.3	1.2	19.9	23.2
AdHCMV--β-gal-C (IM)	5	21.5	21.3	2.4	18.2	23.9
protein-hOb (IP)	10	21.7	21.7	1.7	19.1	24.2
protein-vehicle C (IP)	5	21.6	20.7	1.9	19.7	24.1

The following table compares the maximum response observed in *ob/ob* and lean mice from day 0 to day 23

Treatment	*ob/ob* median change, (% change)	*Ob/?* median change, (% change)
AdHCMV-mOB (IV)	-14.2 (-28.4%)	-2.9 (-13.2%)
AdHCMV-hOB (IV)	-11.1 (-21.3%)	-2.1 (-9.4%)
protein hOB (IP)	-8.9 (-17.3%)	-1.5 (-6.9%)
Controls	-0.4 to 0.1 (-0.7% to 0.2%)	-1.4 to -0.4 (-5.5% to -2.0%)

EXAMPLE 6

Helper-Dependent Constructs

Construction of plasmid pSTK was as follows using standard techniques, known to those of ordinary skill in the art. Bluescript KSII (Stratagene) DNA was cleaved with EcoRV. A double-stranded oligodeoxynucleotide with the restriction sites AscI-AvrII-

FseI-PacI was generated by annealing the single-stranded oligodeoxynucleotides #17302 and #17303:

#17320: 5'-GGC GCG CCC CTA GGG GCC GGC CTT AAT TAA -3' (SEQ.ID.NO:3)

#17303: 5'-TTA ATT AAG GCC GGC CCC TAG GGG CGC GCC-3' (SEQ.ID.NO:4)

The oligodeoxynucleotide was inserted into the EcoRV side of Bluescript KSII using T4 DNA ligase (NEB). The resulting plasmid was called STK2. STK2 was cleaved with BstXI and the BstXI site was made blunt ended using T4-DNA Polymerase (Pharmacia). A double-stranded deoxyoligonucleotide with the restriction sites SwaI-PmeI-SNaBI was generated by annealing the single-stranded oligodeoxynucleotides #17300 and #17301:

#17300: 5'- ATT TAA ATG CCC GCC CGT TTA AAC TAC GTA -3' (SEQ.ID.NO:5).

#17301: 5'- TAC GTA GTT TAA ACG GGC GGG CAT TTA AAT-3' (SEQ.ID.NO:6)

The oligodeoxynucleotide was inserted into the blunt-ended BstXI site using T4-DNA ligase. The resulting plasmid was called STK3.

The left terminus of Adenovirus type 5 was amplified by PCR using oligodeoxynucleotides #23531 and #23532.

#23531: 5'-AGC TTT GTT TAA ACA TCA TCA ATA ATA TAC CTT ATT TTG - 3' (SEQ.ID.NO:7), where the bolded area is a PmeI restriction site and the underlined is Adenovirus type 5 base pairs 1-26.

#23531: 5'- CGA TAA GCT T*GA TAT C*AA AAC GCC AAC TTT GAC CC-3' (SEQ.ID.NO:8) where the bolded areas are a HindIII restriction site, the italic are an EcoRV site, and the underlined is Adenovirus type 5 base pairs 440-421.

The resulting PCR product was cleaved with PmeI and HindIII. STK3 was cleaved with PmeI and HindIII and the Pme/HindIII cleaved PCR product described above was inserted into the PmeI and HindIII sites of STK3 using T4-DNA ligase. The resulting plasmid was called STK31.

The right terminus of Adenovirus type 5 was amplified by PCR using oligodeoxnucleotides #23531 *supra*, and #24147:

#24147: 5'-CGA TAA GCT T*GA TAT* CAC TCC GCC CTA AAA CCT ACG -3' (SEQ.ID.NO:9), wherein the bolded area is a HindIII restriction site, the italic is an EcoRV site, an the underlined area is Adenovirus type 5 base pairs 35818-35837.

The resulting PCR product was cleaved with PmeI and HindIII.

STK3 was cleaved with PmeI and HindIII and the PmeI/HindIII cleaved PCR product described above was insewrted using T4-DNA ligase. The resulting plasmid was called STK3-23531/24147.

STK31 was cleaved with EcoO109I The EcoO109I site was made blunt-ended using the Klenow fragment of DNA Polymerase I (Pharmacia). Plasmid STK3-23531/24147 was cleaved with SnaBI and EcoRV. The resulting SnaBI-EcoRV fragment containing the right terminus of Adenovirus type 5 was inserted into the EcoO109I site of STK31. The resulting plasmid was called STK42.

A cosmid containing part of the hypoxanthine guanine phosphoribosyltransferase (HPRT) gene (U72D8) was cleaved with EclXI and PmeI. EclXI cleaves at bp 1799 and PmeI at bp 17853 of the sequences which is deposited in the GenBank database (Locus: Human HPRT gene [HUMHPRTB]; gb:humhprtb). STK3 was cleaved with PmeI and EclXI. The 16054 bp EclXI/PmeI HPRT fragment from the HPRT gene containing cosmid was inserted into the PmeI and EclXI sites of STK3. The resulting plasmid was called STK55.

STK42 was cleaved with HincII. STK55 was cleaved with PmeI and SalI. The SalI site was made blunt-ended using the Klenow fragment of DNA Polymerase I. The resulting fragment that contained the 16054 bp EclXI/PmeI HPRT fragment was inserted into the HincII site of STK42. The resulting plasmid was called STK68.

STK68 was cleaved with AscI. AscI was made blunt-ended using the Klenow fragment of DNA Polymerase I. The cosmid C346 (Andersson et al. 1995 *DNA Seq.* 5: 219-223, which is hereby incorporated by reference) was cleaved with HindIII and the ends were made blunt-ended using the Klenow fragment of DNA Polymerase I.

HindIII cleaves at bp 12421 and 21484 of the sequence that is deposited in the GenBank database (Locus: HUMDXS455A; gb:L31948). The 9063 bp HindIII fragment of C346 was inserted into the AscI site of STK68. The resulting plasmid was designated pSTK120.

After cloning of the leptin expression cassettes described previously into pSTK120, 10 μg of the recombinant constructs were digested with PmeI enzyme, and 5 μg of digested DNA was transfected into 293-cre4 cells monolayer/well in a 6 well plate using Lipofectamine (GIBCO) following the manufacturer's instructions. 24 hours post-transfection, the cells were infected with approximately 5 X 10^5 plaque forming units (pfu) of helper virus AdLC8clucl. Cells were collected after cytopathic effect (CPE) was observed, which took approximately 3-5 days. Lysate was used to infect 6 cm plates of 293-cre4 monolayers. Plates were infected with 1 ml lysate and 1 ml media (MEM-α supplemented with 10% serum 1 X pen/strep, 1 X L-glutamine and sodium bicarbonate), followed 24 hours later with 0.1-1 X 10^6 pfu of the helper virus. After CPE (approximately 4-5 days), lysate was collected and used to infect 10 cm plates of 293-cre4 cells, the same process was followed, infection in 10 cm plates used 2 ml lysate, 8 ml media and 1-5 X 10^6 helper virus (added 24 hours post lysate infection). CPE collection was followed and lysate was used to infect 15 cm plates of 293-cre4 cells (approximately 1 X 10^7 cells). 5 ml cells plus 15 ml media was used with 0.1-1 X 10^7 pfu helper was added 24 hours post-lysate collection. After CPE collected lysate was used to infect twenty 15 cm plates of 293 cre4 cells, this step (passage 5) is the first passage used for cesium banding and virus purification.

Cesium banding and virus purification was identical to the process used to purify the first generation virus. Supernatant from passage 5 was also used to further propagate the virus. Occasionally two cesium bands were observed, the lower band was the helper dependent virus. Virus was dialyzed and as was the case with the first generation, titered on regular 293 cells to determine the level of contaminating helper viruses which are capable of plaque formation.

The helper dependent virus is not capable of forming plaques; therefore to estimate levels of rescued virus, optical density (OD) readings were made. It is estimated that the infective particles are approximately 10 to 100-fold lower that the estimated particle number measured by optical density. Also, comparisons between the viral vectors described in Examples 1-5 and the helper dependent leptin gene expression were used to estimate particle number. Yield was approximately 8×10^{12} particles (2×10^{12}/ml). For semi-quantitation of infectious particles, COS cells were infected with 10 µl of HD-leptin or with Ad-leptin at a moi of 10 or 15. Cells were washed 30 minutes post-infection and serum-free media was added. 100 µl aliquots of media were collected from infected plates at 24, 30, 48 and 54 hours post-treatment, and compared by western blot analysis for leptin protein levels, using a polyclonal anti-leptin antibody (Santa Cruz Biotech) (See Figure 25B). Leptin was detected as a single band at approximately 16 kD. The HD-leptin mediated expression was equivalent to the 15 moi infected plates, and based on the pfu titer of Ad-leptin, the estimated titer was approximately $1\text{-}2 \times 10^{10}$/ml with a particle to infectious unit ratio of approximately 1:100.

EXAMPLE 7

Ob/ob (C57BL/J6-ob/ob) and litter mates, homozygous normal lean mice, age and sex matched (females) were purchased from Jackson Laboratories (Bar Harbor, ME) for the use in this study. Animals were free of all common murine pathogens. Eight to twelve weeks old mice (*ob/ob* approximately 70 gm and lean approximately 28 gm) were re-distributed based on equal representation of weight and caged in groups of 5 on day 0, immediately preceding treatment. After a series of baseline blood samples were obtained by tail incision from conscious mice, animals were divided into 4 groups and treated by tail vein injection of a single 100 µl aliquot of $1\text{-}2 \times 10^{11}$ particles of HD-leptin, Ad-leptin, Ad-βgal (control) or dialysis buffer (control). Body weight and food intake were measured daily and blood was collected 2-3

times weekly, pre- and post-treatment. Animals were killed by carbon dioxide inhalation and organs removed for immunohistochemistry and RNA analysis. All animals used in this study were maintained in accordance with the "Guide for the Care Use of Laboratory Animals" (DHHS Publication No.(NIH) 85-23, revised 1996). The protocol was approved by the Institutional Animal Care and Use Committee, Merck Research Laboratories, West Point, PA.

Blood samples were obtained by tail incision, and collection into heparinized microhematocrit tubes (VWR) every 2-3 days during the course of the study. Tubes were centrifuged at 13,700 g for 2 minutes and hematocrit values were monitored. Plasma was collected for measurement of glucose and leptin levels. Glucose levels were measured using the Kodak Ektachem DT slides (Eastman Kodak Co.). Leptin and insulin levels were measured by an RIA assay performed by Linco Research, Inc.

WHAT IS CLAIMED IS:

1. A method of treating a condition caused at least in part by an insufficient production of a peptide or protein hormone in a mammal comprising administration of a viral vector comprising a gene construct encoding the peptide or protein hormone.

2. A method according to Claim 1 wherein the hormone is selected from the group consisting of: leptin, insulin, calcitonin, erythropoietin, growth hormone, an interferon, interleukin-2, a hemophilia factor, a vascular endothelial growth factor, granulocyte-macrophage colony stimulating factor, and alpha 1 anti-trypsin.

3. A method according to Claim 1 wherein the viral vector is a adenoviral vector or a helper-dependent adenoviral vector.

4. A method according to Claim 3 wherein the viral vector is administered at a dose of at least about 5×10^6 plaque forming units (pfu) per gram of body weight of the mammal.

5. A method of treating obesity, lowering serum glucose levels or lowering serum insulin levels in a mammal in need of such therapy comprising delivering a gene encoding leptin to the mammal; wherein transcription and translation of the gene encoding leptin occurs *in vivo*.

6. A method according to Claim 5 wherein the gene encoding leptin is delivered in a viral vector.

7. A method according to Claim 6 wherein the vector is an adenovirus or a helper dependent adenovirus.

8. A transgenic non-human mammal or progeny thereof which expresses a leptin transgene.

9. A mammal according to Claim 8 which is a mouse.

10. A mouse according to Claim 8 which is an *ob/ob* mouse.

11. A helper dependent adenoviral vector comprising:
a) a first segment of adenovirus DNA;
b) a first segment of stuffer DNA;
c) a transgene construct comprising a leptin transgene;
d) a second segment of stuffer DNA; and
e) a second segment of adenovirus DNA;
wherein no adenoviral proteins are expressed in a host cell.

12. A viral vector according to Claim 11 wherein the first and second adenovirus DNA segments together comprise adenovirus inverted terminal repeats that comprise a viral origin of replication and packaging signals.

13. A viral vector according to Claim 12 wherein the stuffer DNA and the transgene construct is at least about 28 kb.

14. A viral vector according to Claim 13 which is pSTK120.

15. A mammalian cell comprising a vector according to Claim 11.

16. A method of determining if a compound possesses leptin-modulating activity *in vivo* comprising administering the compound to a mammal of Claim 8.

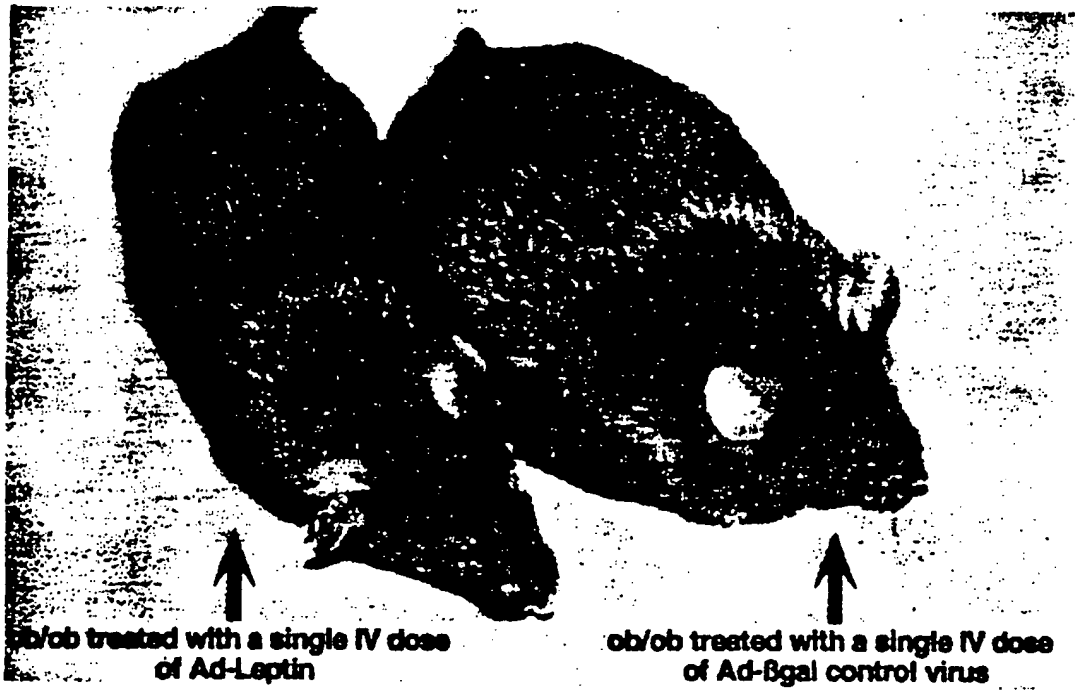

ob/ob treated with a single IV dose of Ad-Leptin
ob/ob treated with a single IV dose of Ad-βgal control virus

FIG. 1

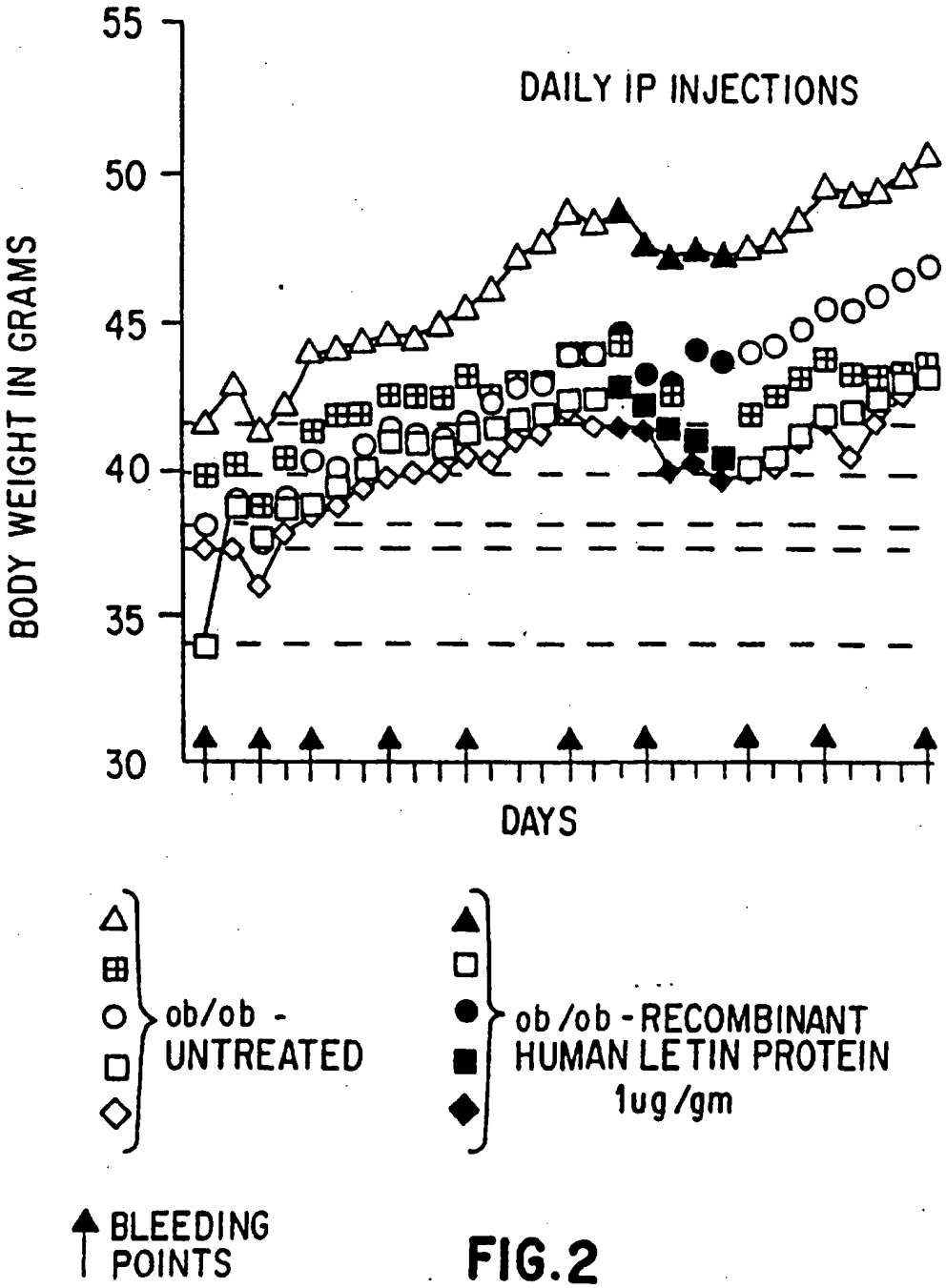

55
50
45
40
35
30
BODY WEIGHT IN GRAMS
DAILY IP INJECTIONS
DAYS
ob/ob - UNTREATED
ob/ob - RECOMBINANT HUMAN LETIN PROTEIN 1ug/gm
BLEEDING POINTS

FIG.2

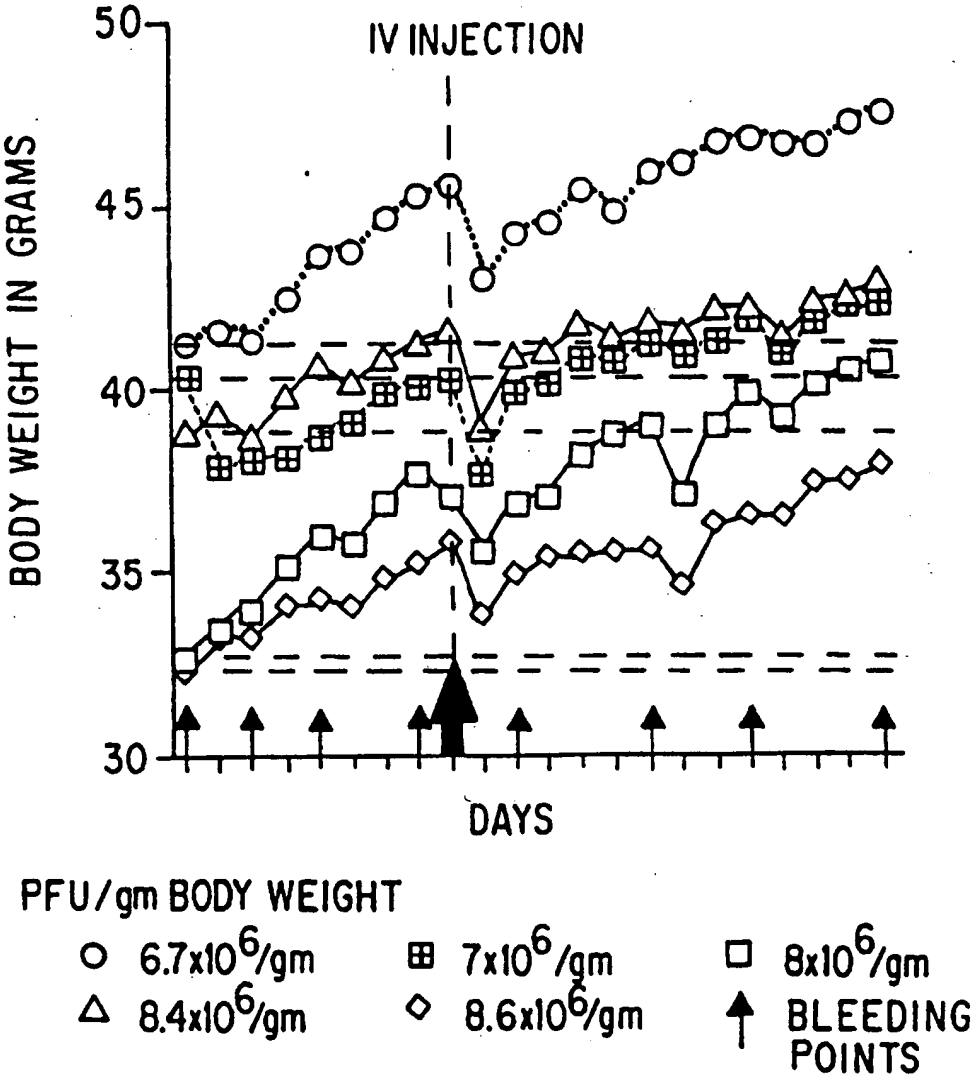

50
45
40
35
30
IV INJECTION
BODY WEIGHT IN GRAMS
DAYS
PFU/gm BODY WEIGHT
6.7x10^6/gm
7x10^6/gm
8x10^6/gm
8.4x10^6/gm
8.6x10^6/gm
BLEEDING POINTS

FIG.3

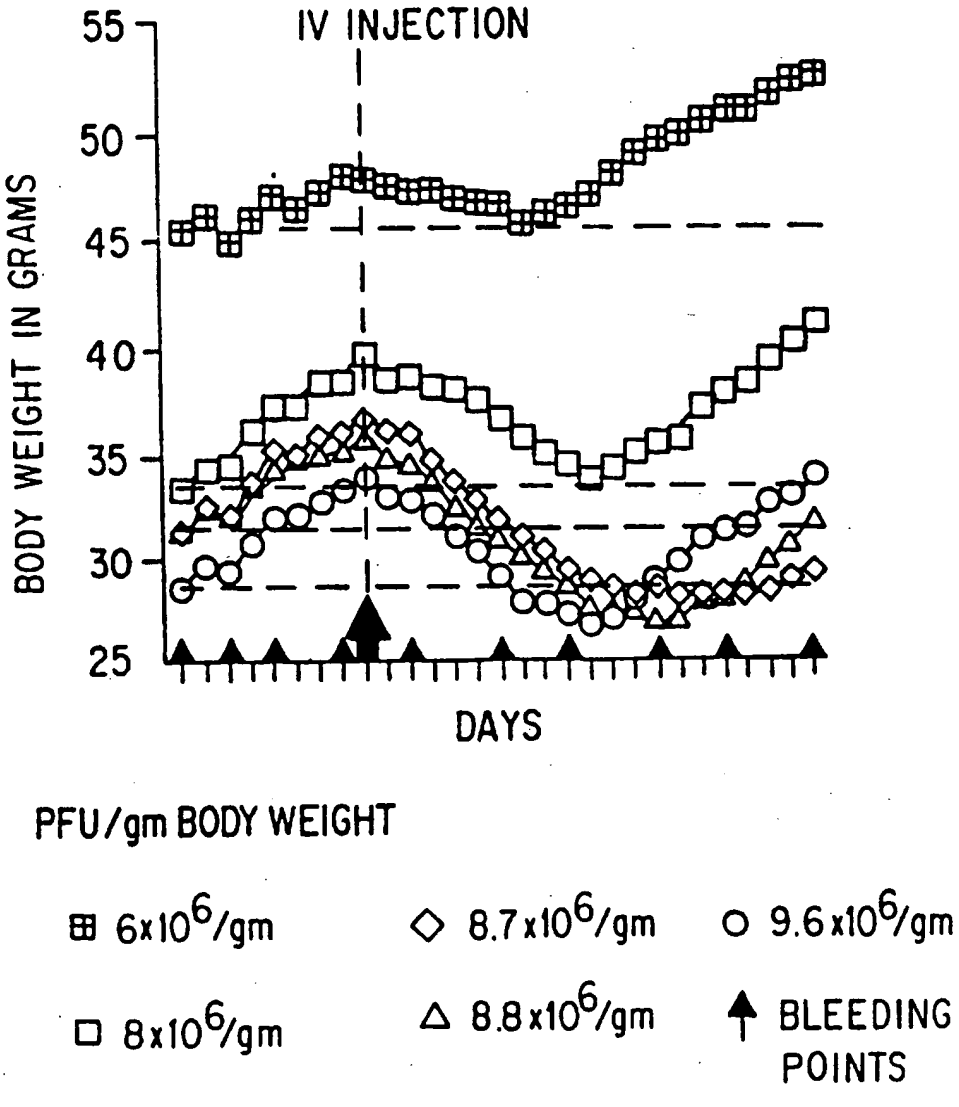

IV INJECTION
55
50
45
40
35
30
25
BODY WEIGHT IN GRAMS
DAYS
PFU/gm BODY WEIGHT
6x10^6/gm
8.7x10^6/gm
9.6x10^6/gm
8x10^6/gm
8.8x10^6/gm
BLEEDING POINTS

FIG.4

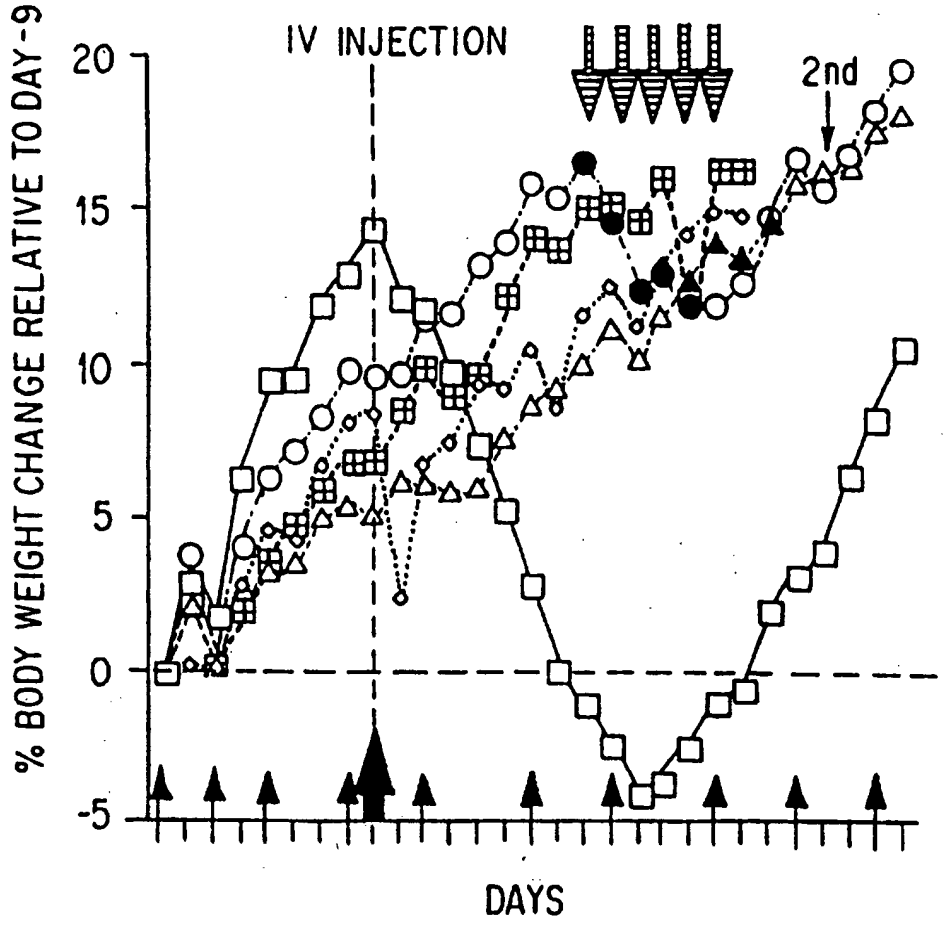

IV INJECTION
2nd
% BODY WEIGHT CHANGE RELATIVE TO DAY -9
20
15
10
5
0
-5
DAYS
MEANob/ob-Adhob
MEANob/ob-noRx
MEANdb/db-Adhob/noRx
MEANob/ob-AdβgaI
MEANob/ob-LEPTIN PROTEIN
MEANdb/db-LETIN PROTEIN
MEANdb/db AdβgaI
BLEEDING POINTS
IP INJECTION OF RECOMBINANT HUMAN LEPTIN (1ug/gm BODY WT)

FIG.5

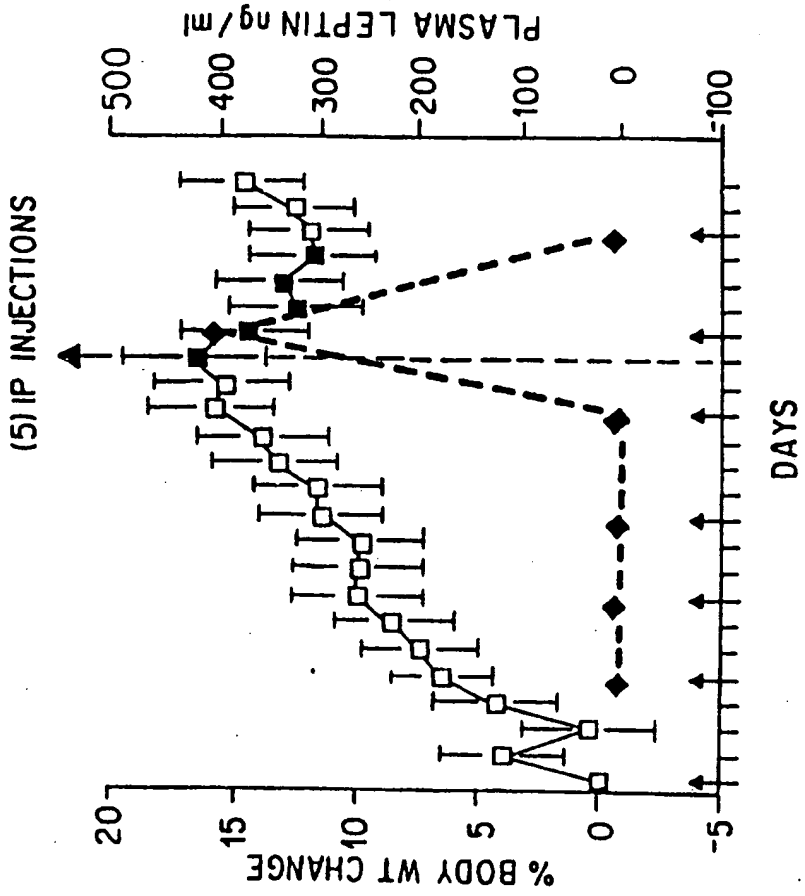

PLASMA LEPTIN ng/ml
500
400
300
200
100
0
-100
(5) IP INJECTIONS
DAYS
% BODY WT CHANGE
20
15
10
5
0
-5

FIG.6B

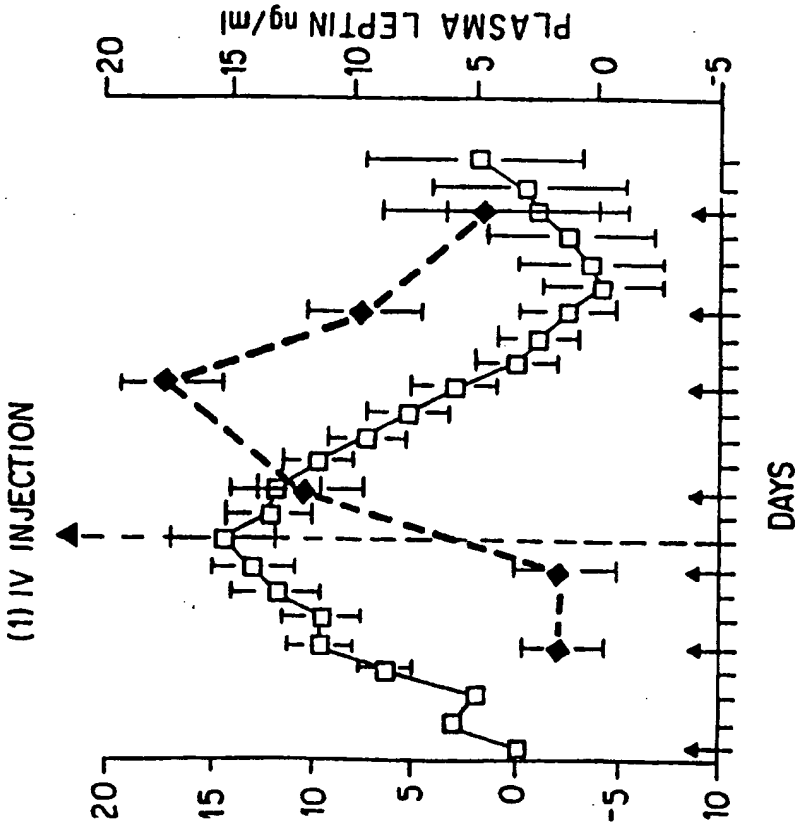

PLASMA LEPTIN ng/ml
20
15
10
5
0
-5
(1) IV INJECTION
DAYS
20
15
10
5
0
-5
10

FIG.6A

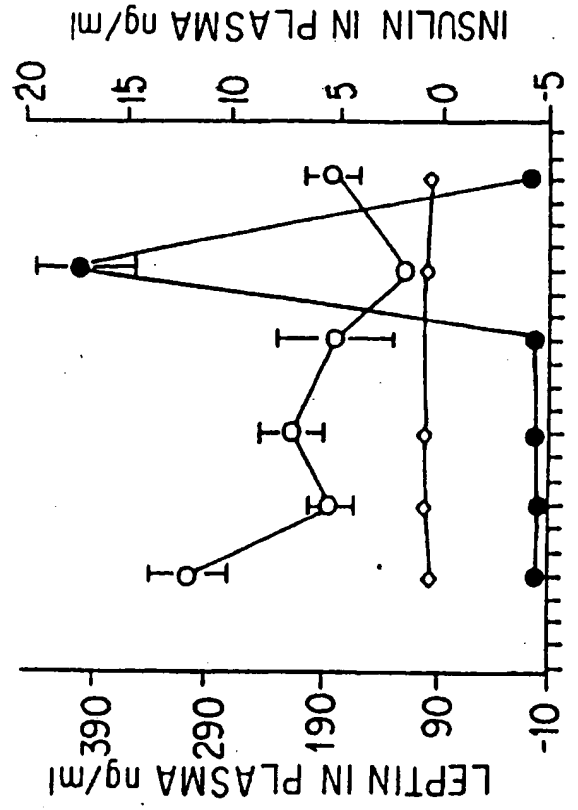
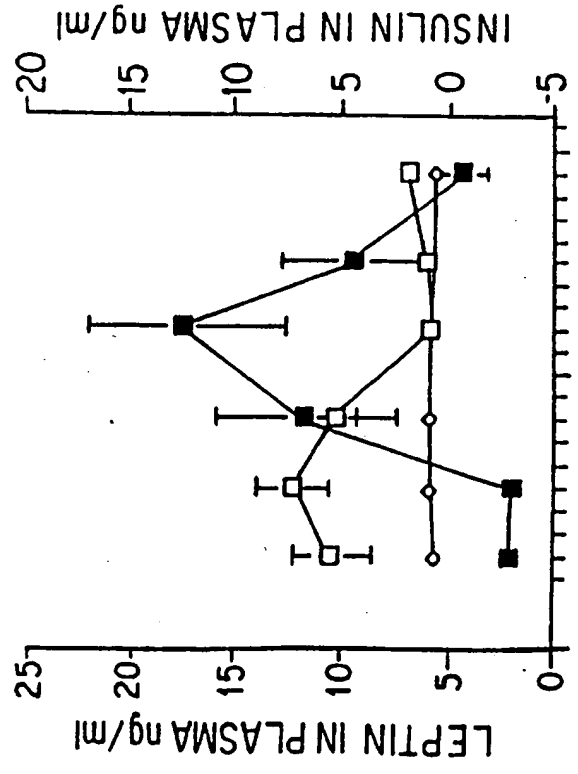


FIG.7B



- ● HUMAN LEPTIN IN ob/ob TREATED WITH 1ug/gm RECOMBINANT PROTEIN DAILY FOR 5 DAYS
- ■ HUMAN LEPTIN IN ob/ob TREATED WITH A SINGLE INJECTION OF 2.75x108 pfu OF VIRAL VECTOR
- ◇ LEAN INSULIN LEVELS
- □ INSULIN IN ADENO-TREATED ob/ob
- ○ INSULIN IN RECOM. LEPTIN TREATED ob/ob

FIG.7A

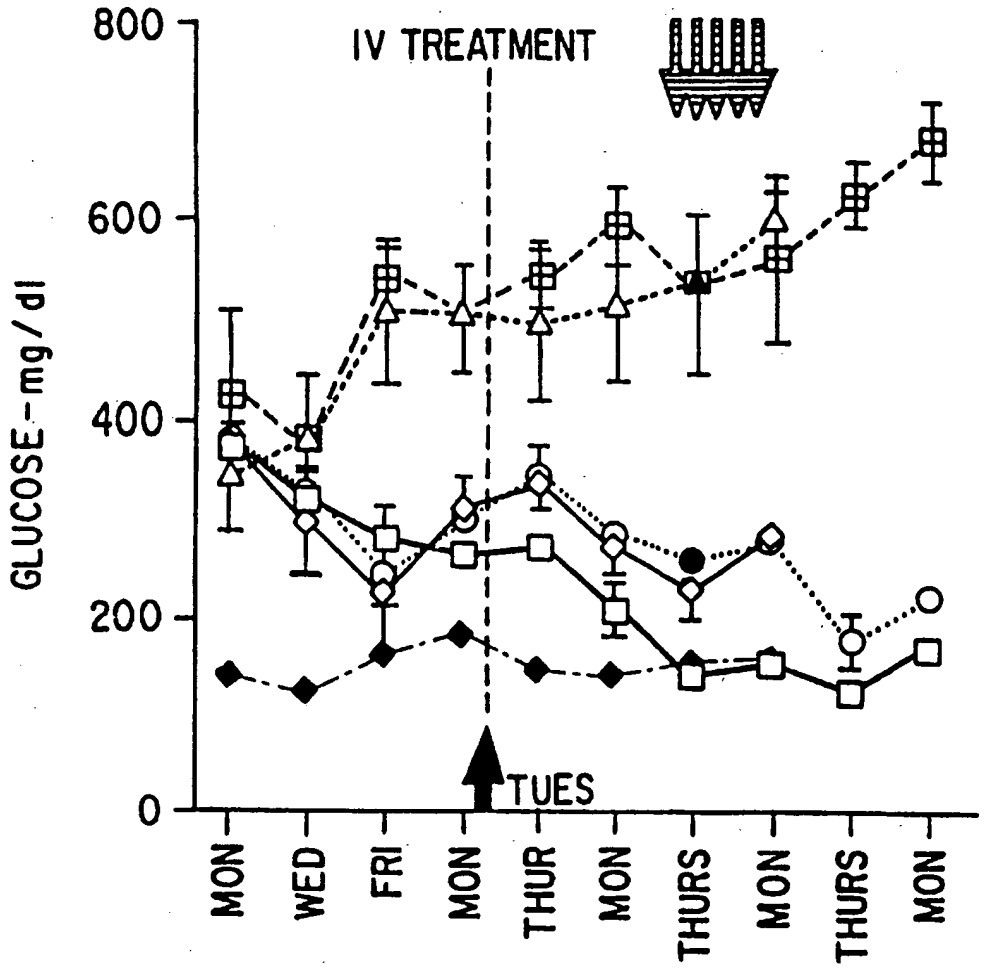

800
600
400
200
0
GLUCOSE-mg/dl
IV TREATMENT
TUES
MON
WED
FRI
MON
THUR
MON
THURS
MON
THURS
MON
ob/ob-Adhob-mean
ob/ob-noRx-mean
db/db-Adhob-noRx-mean
db/db-AdβgaI
ob/ob-AdβgaI-mean
ob/ob-LEPTIN PROTEIN-MEAN
db/db-LEIN PROTEIN-MEAN
LEAN MEAN
IP INJECTION OF RECOMBINANT
HUMAN LEPTIN (1ug/gm BODY WT)

FIG. 8

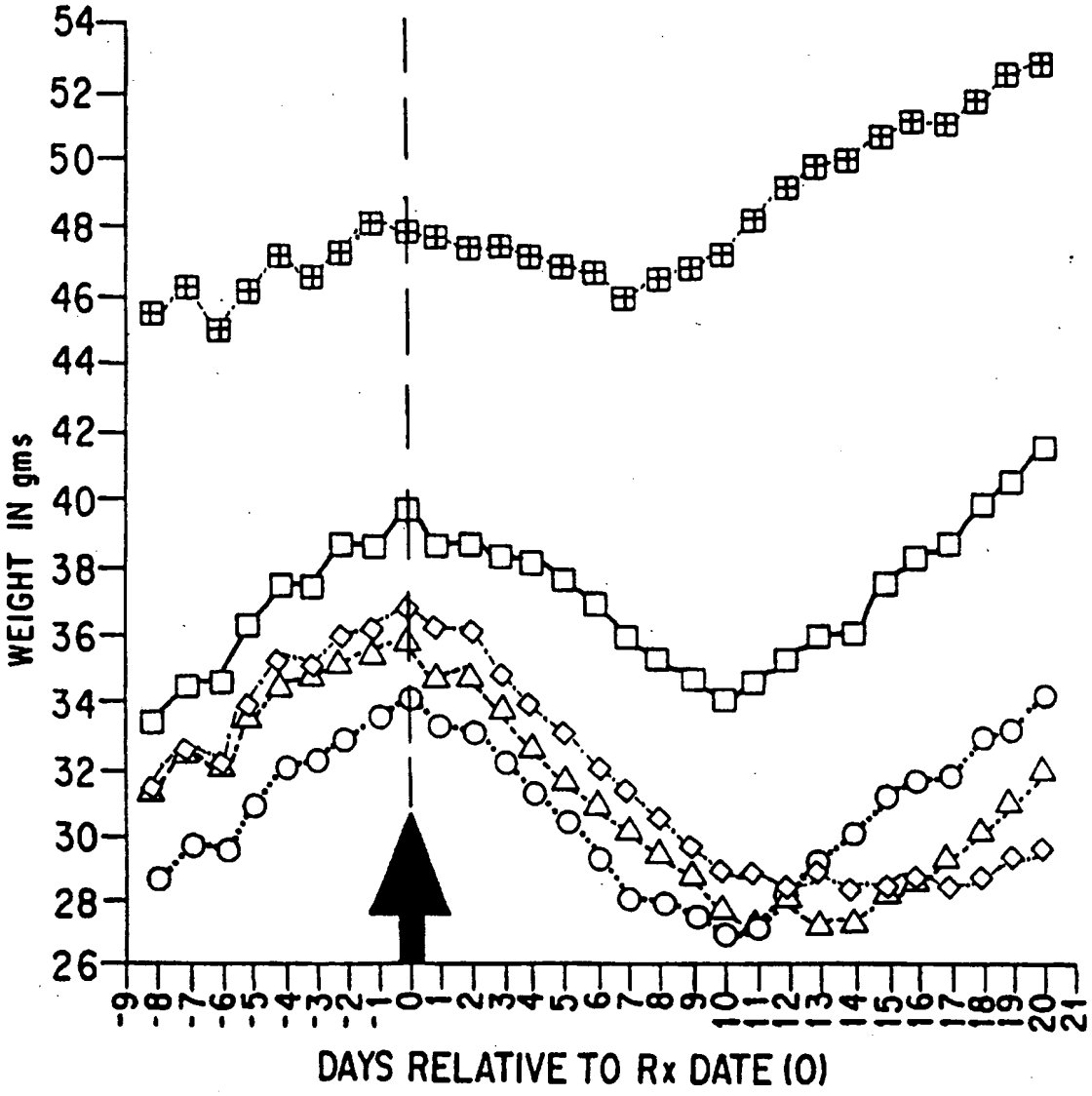

WEIGHT IN gms
54
52
50
48
46
44
42
40
38
36
34
32
30
28
26
-9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
DAYS RELATIVE TO Rx DATE (0)

FIG.9A

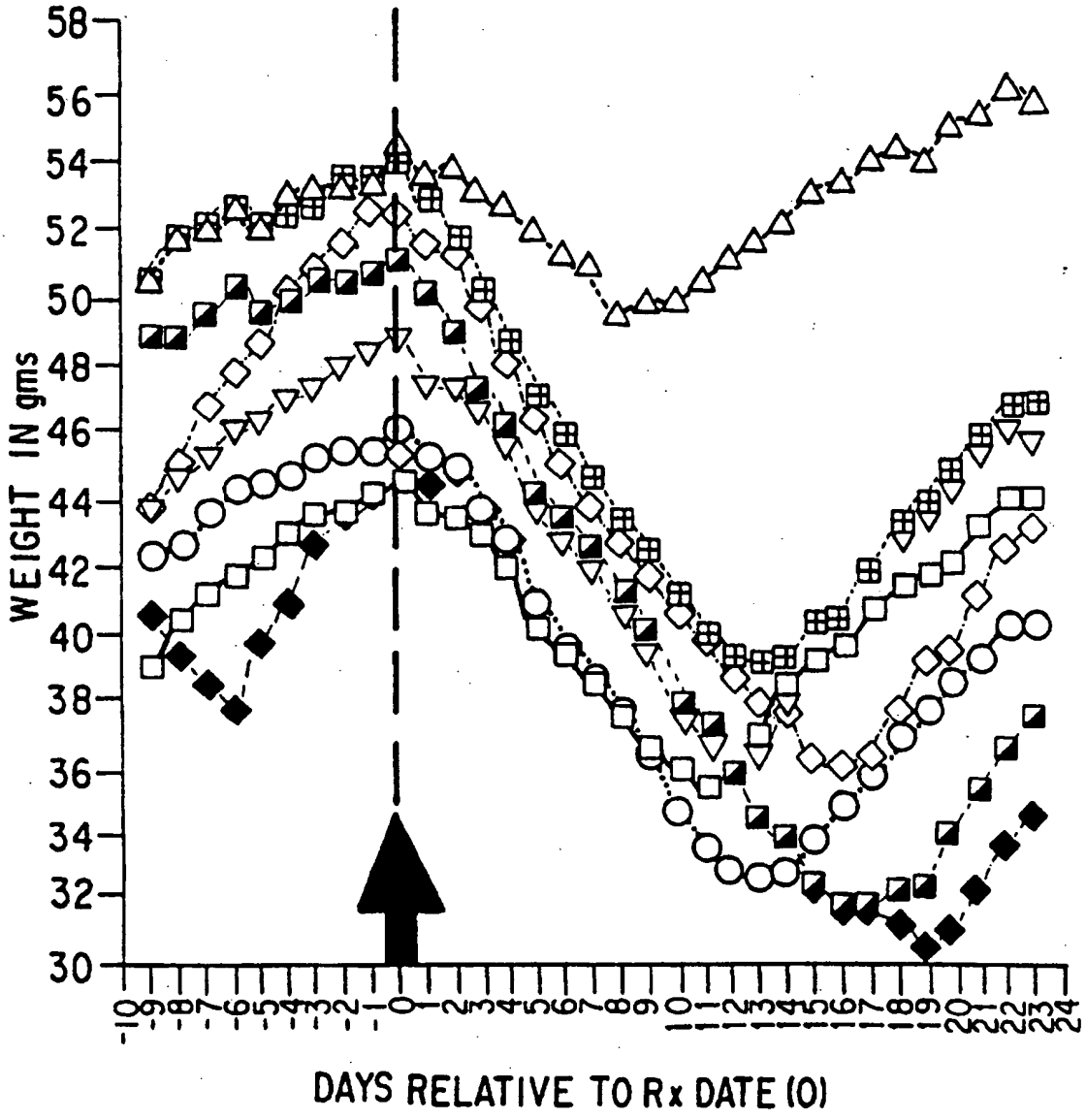

WEIGHT IN gms
58
56
54
52
50
48
46
44
42
40
38
36
34
32
30
DAYS RELATIVE TO Rx DATE (0)

FIG.9B

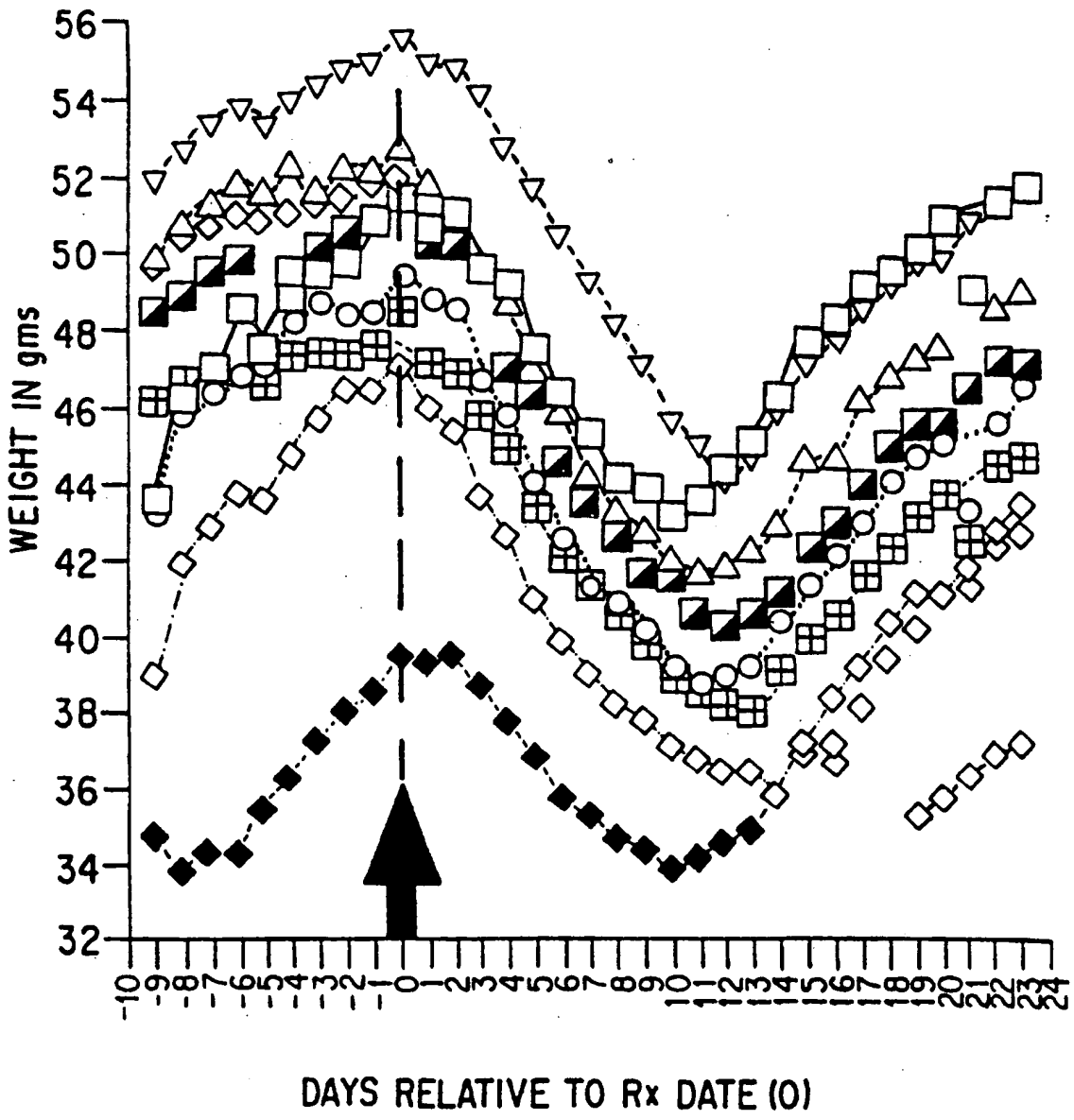

56
54
52
50
48
46
44
42
40
38
36
34
32
WEIGHT IN gms
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
DAYS RELATIVE TO Rx DATE (0)

FIG.9C

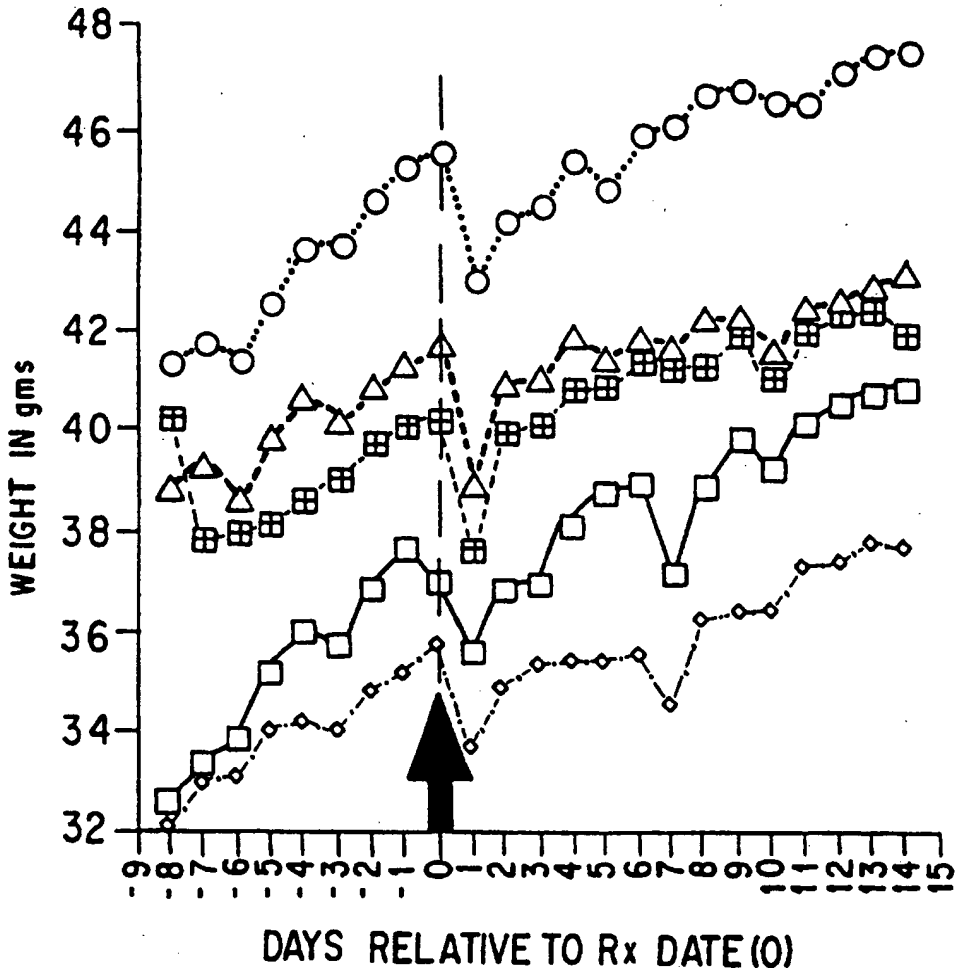

48
46
44
42
40
38
36
34
32
WEIGHT IN gms
-9 -8 -7 -6 -5 -4 -3 -2 -1 0 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS RELATIVE TO Rx DATE (0)

FIG.10A

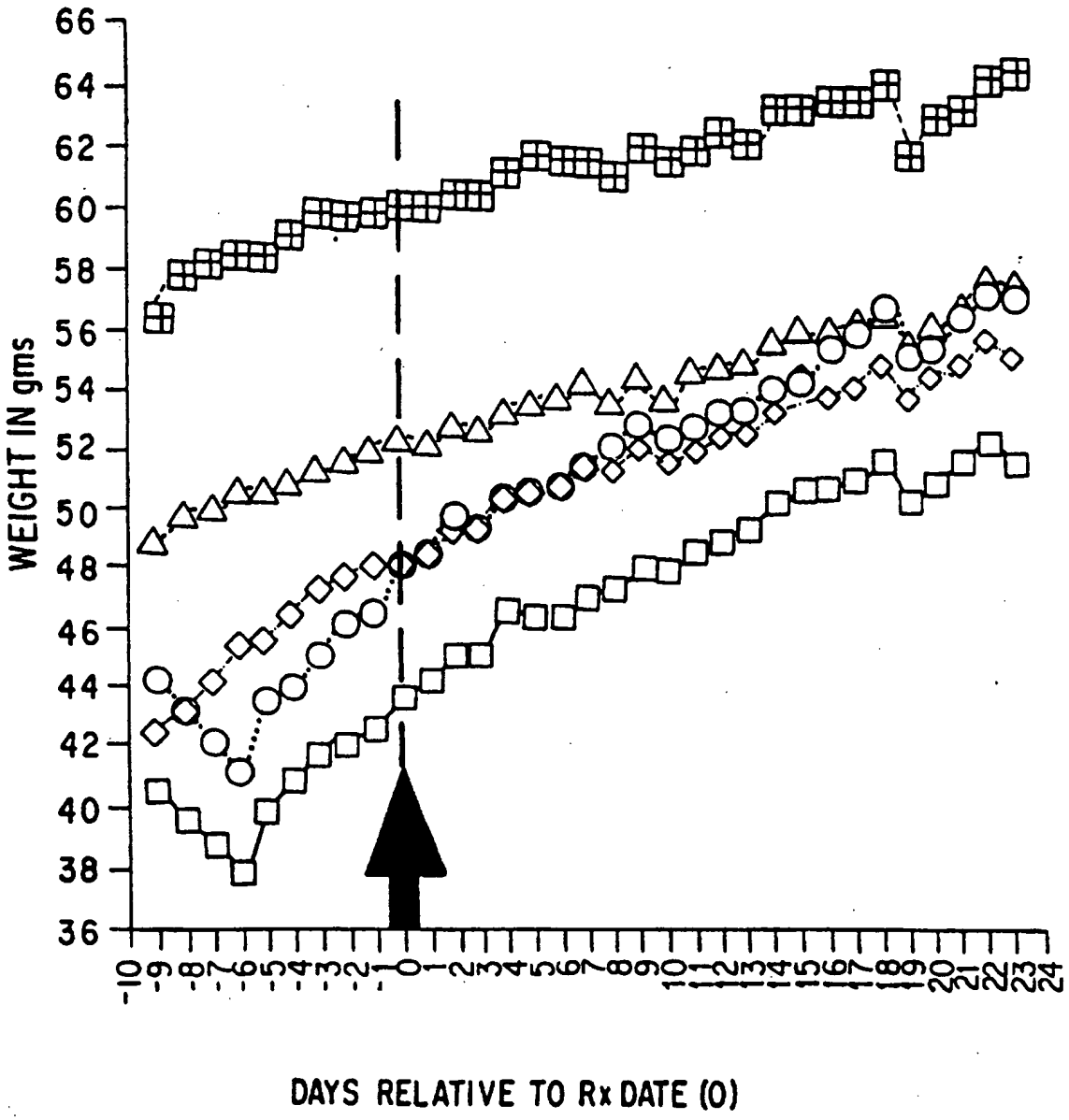

WEIGHT IN gms
66
64
62
60
58
56
54
52
50
48
46
44
42
40
38
36
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
DAYS RELATIVE TO Rx DATE (0)

FIG.10B

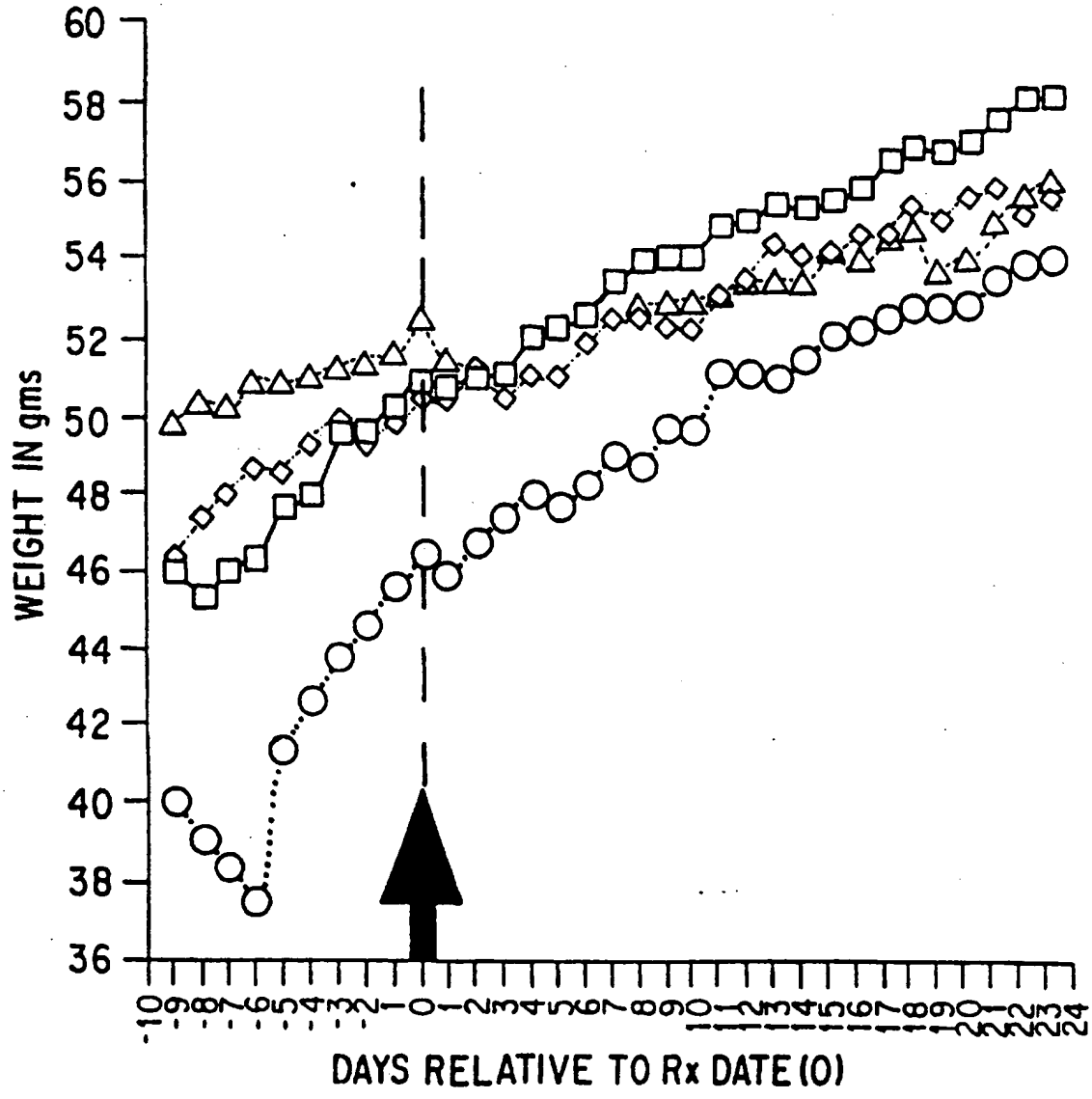

60
58
56
54
52
50
48
46
44
42
40
38
36
WEIGHT IN gms
DAYS RELATIVE TO Rx DATE (0)

FIG.10C

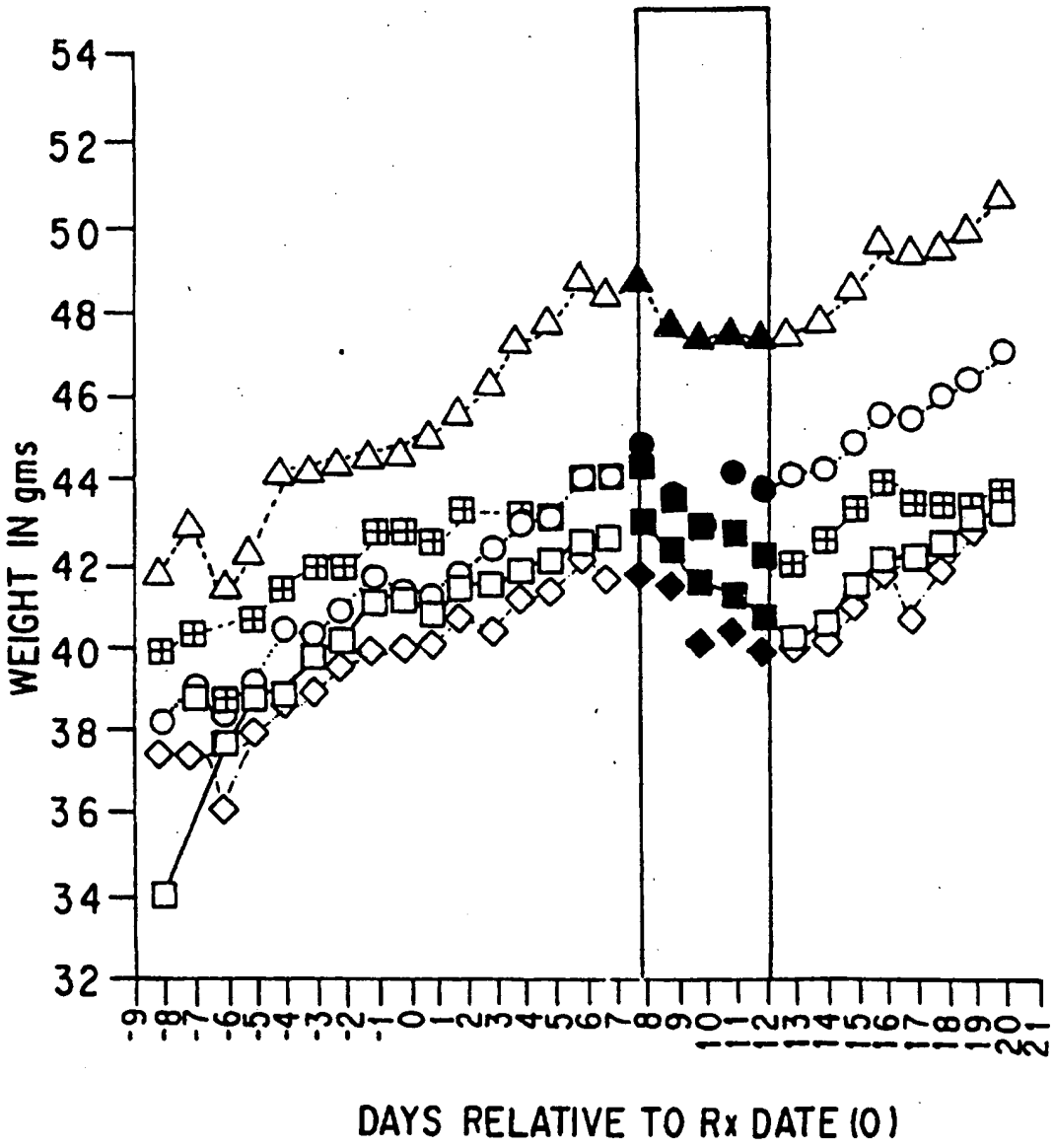

54
52
50
48
46
44
42
40
38
36
34
32
WEIGHT IN gms
-9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
DAYS RELATIVE TO Rx DATE (0)

FIG. 11A

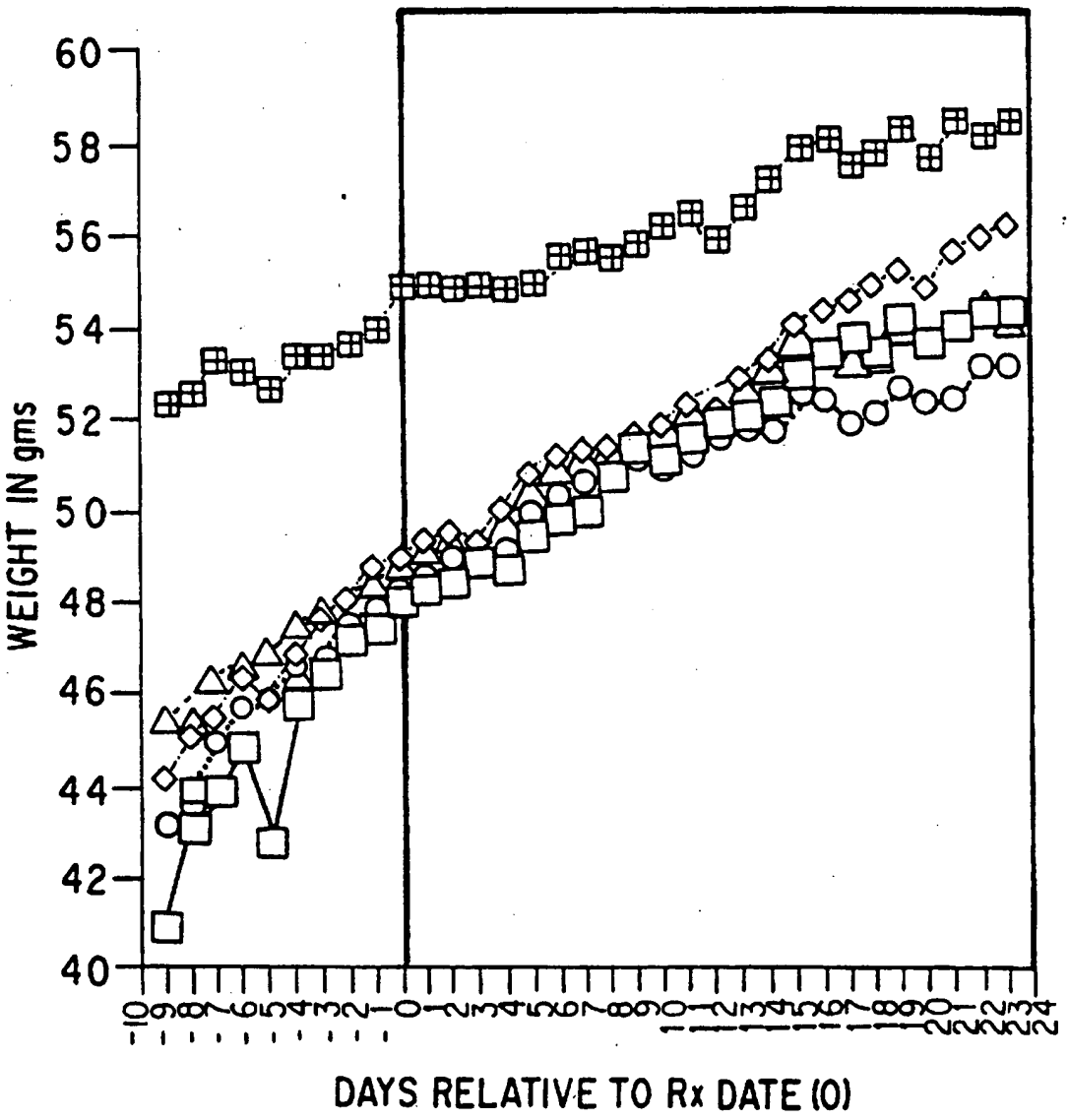

60
58
56
54
52
50
48
46
44
42
40
WEIGHT IN gms
DAYS RELATIVE TO Rx DATE (0)

FIG.11B

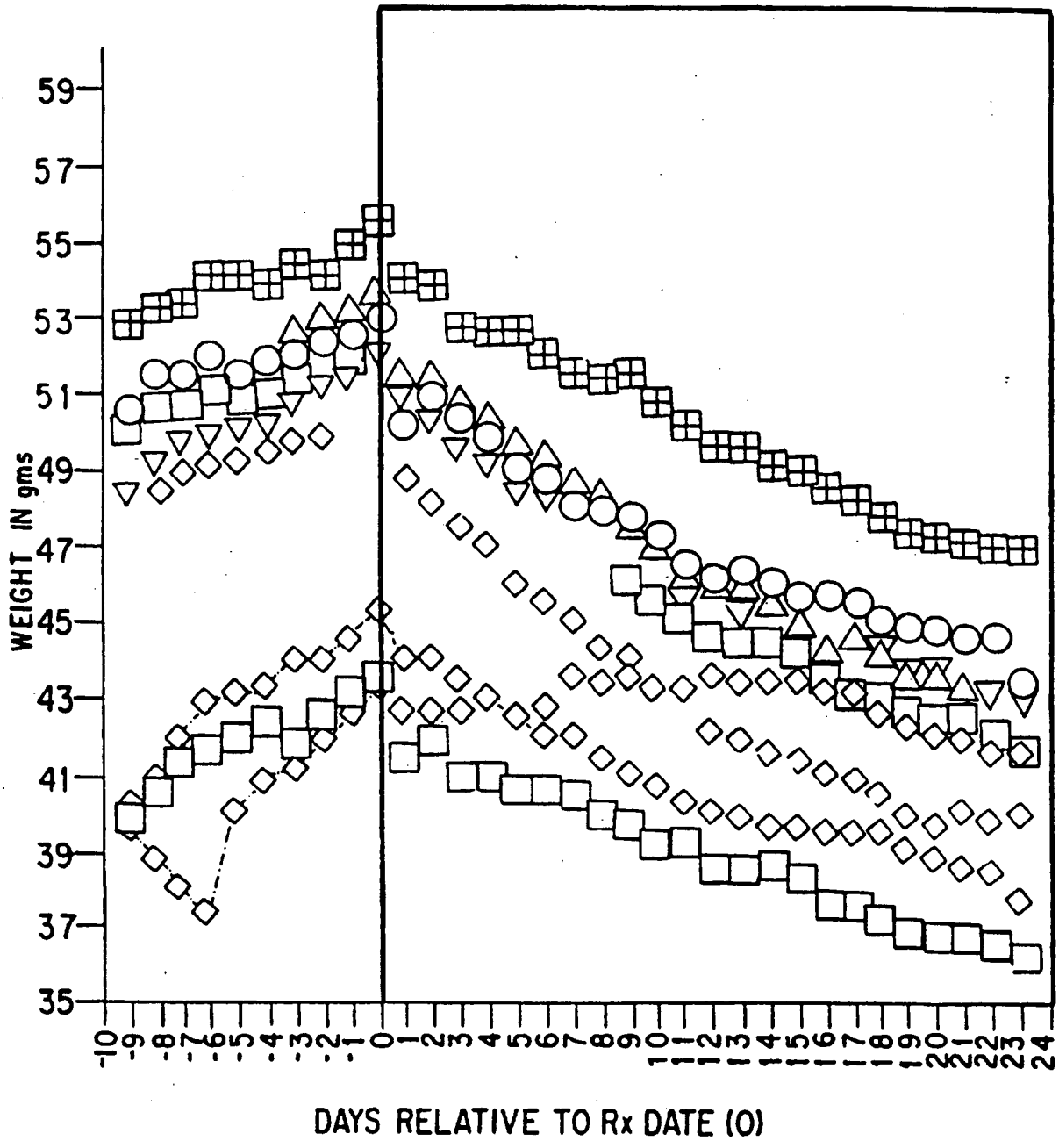

WEIGHT IN gms
59
57
55
53
51
49
47
45
43
41
39
37
35
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
DAYS RELATIVE TO Rx DATE (0)

FIG.11C

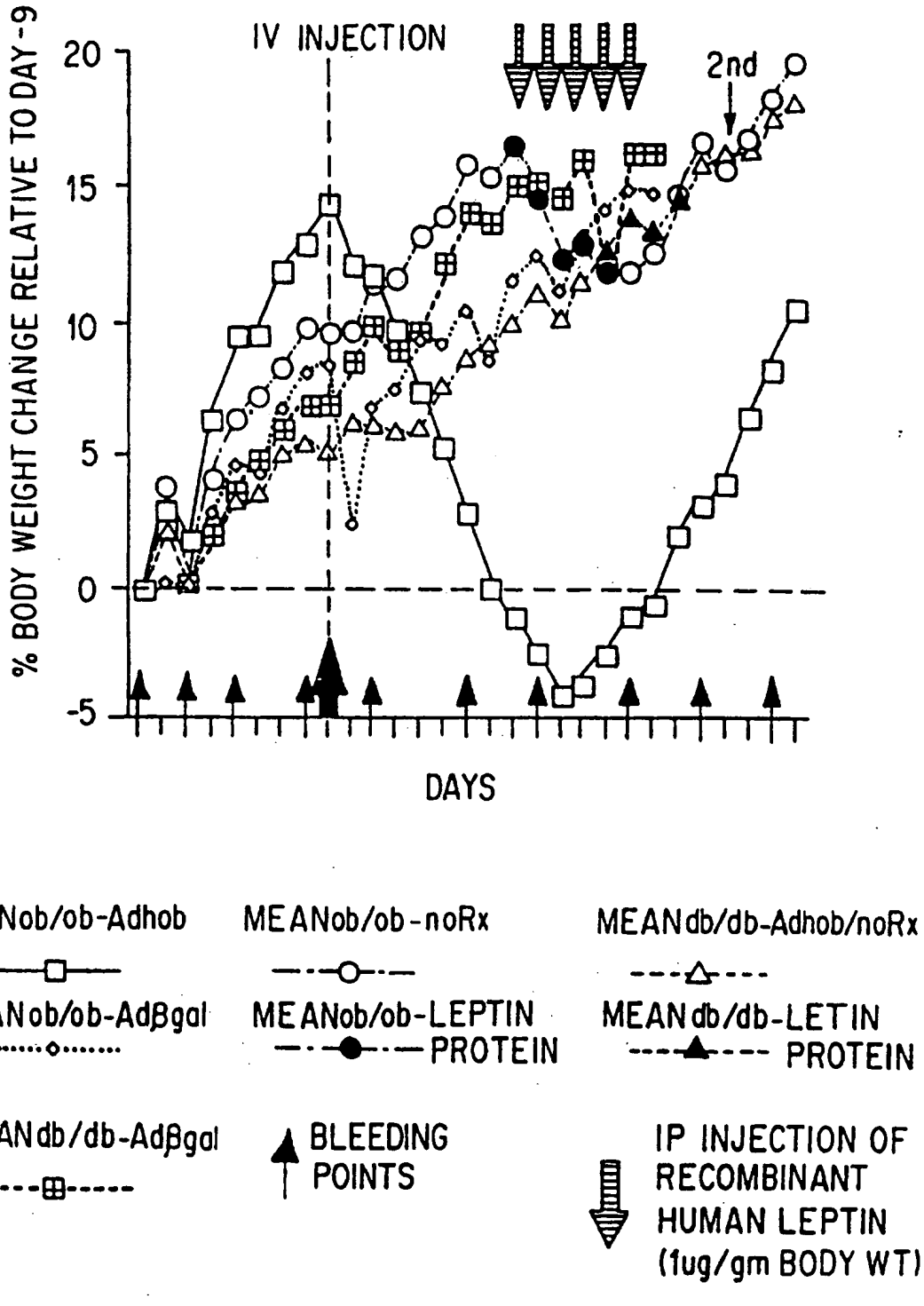

IV INJECTION
2nd
% BODY WEIGHT CHANGE RELATIVE TO DAY -9
20
15
10
5
0
-5
DAYS
MEANob/ob-Adhob
MEANob/ob-noRx
MEANdb/db-Adhob/noRx
MEANob/ob-AdβgaI
MEANob/ob-LEPTIN PROTEIN
MEANdb/db-LETIN PROTEIN
MEANdb/db-AdβgaI
BLEEDING POINTS
IP INJECTION OF RECOMBINANT HUMAN LEPTIN (1ug/gm BODY WT)

FIG. 12

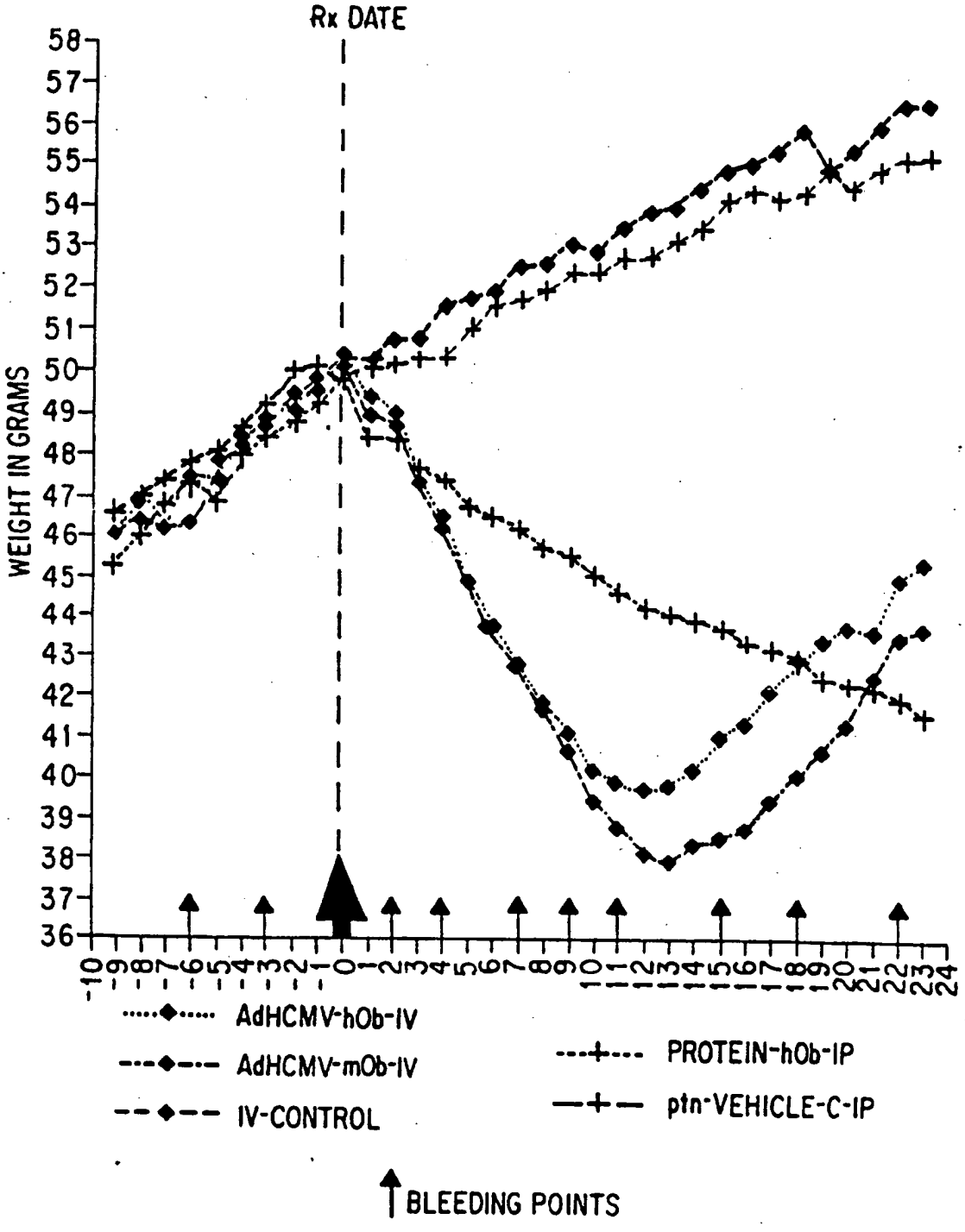

Rx DATE
WEIGHT IN GRAMS
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
-10 -9 -8 -7 -6 -5 -4 -3 -2 -0 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9 20 21 22 23 24
AdHCMV-hOb-IV
AdHCMV-mOb-IV
IV-CONTROL
PROTEIN-hOb-IP
ptn-VEHICLE-C-IP
BLEEDING POINTS

FIG.13

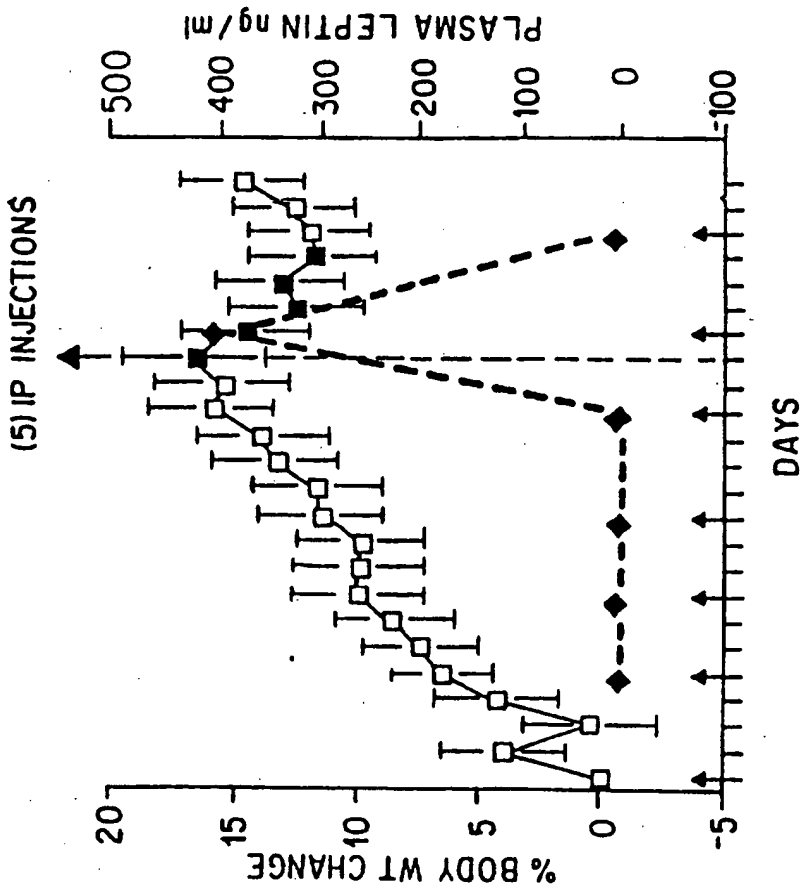


FIG.14B

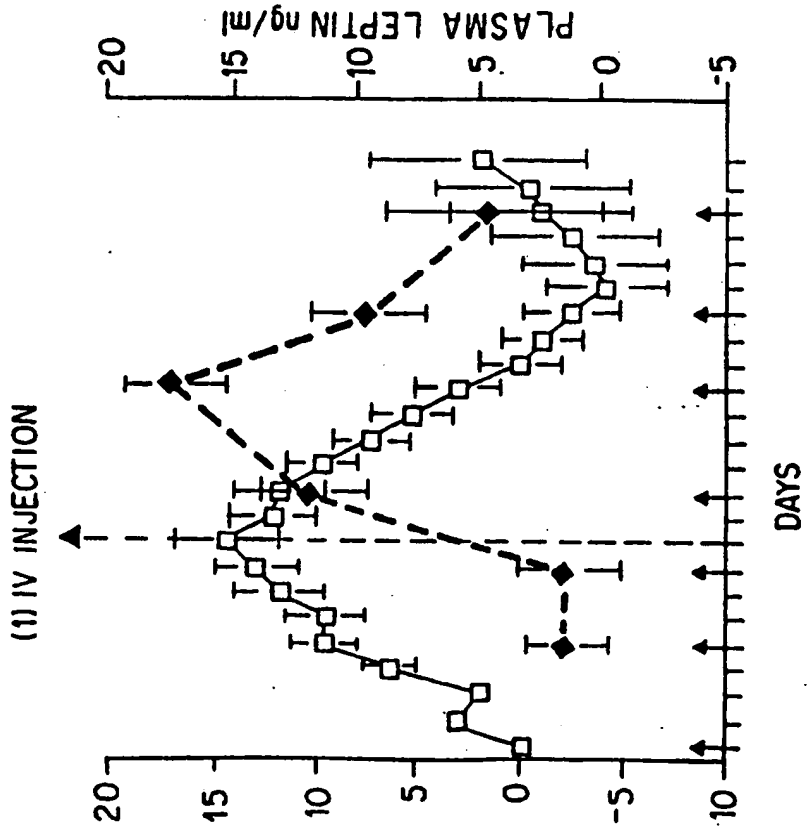


FIG.14A

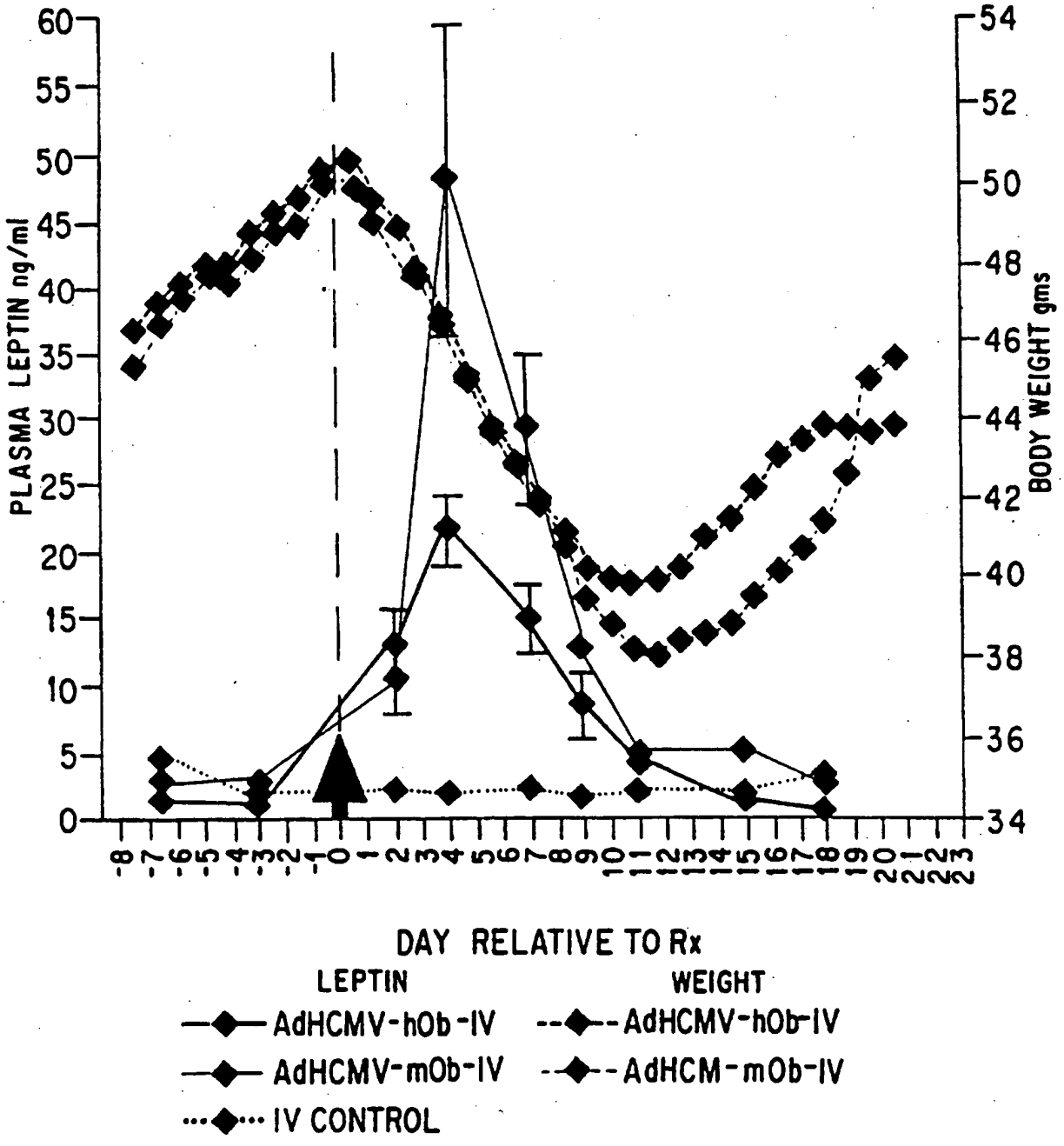

PLASMA LEPTIN ng/ml
BODY WEIGHT gms
DAY RELATIVE TO Rx
LEPTIN
WEIGHT
AdHCMV-hOb-IV
AdHCMV-hOb-IV
AdHCMV-mOb-IV
AdHCM-mOb-IV
IV CONTROL

FIG.15A

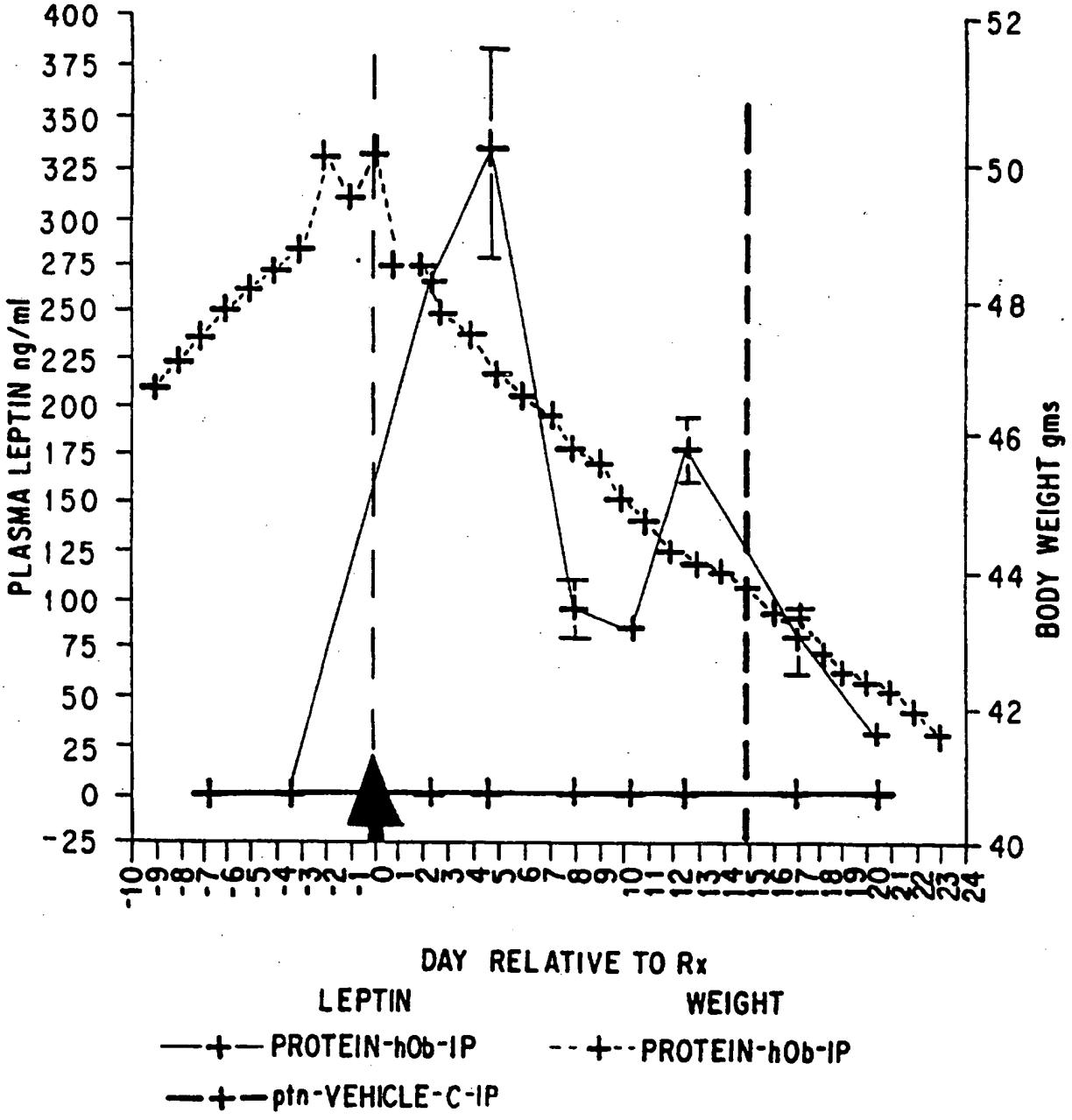

PLASMA LEPTIN ng/ml
BODY WEIGHT gms
DAY RELATIVE TO Rx
LEPTIN
WEIGHT
PROTEIN-hOb-IP
PROTEIN-hOb-IP
ptn-VEHICLE-C-IP

FIG. 15B

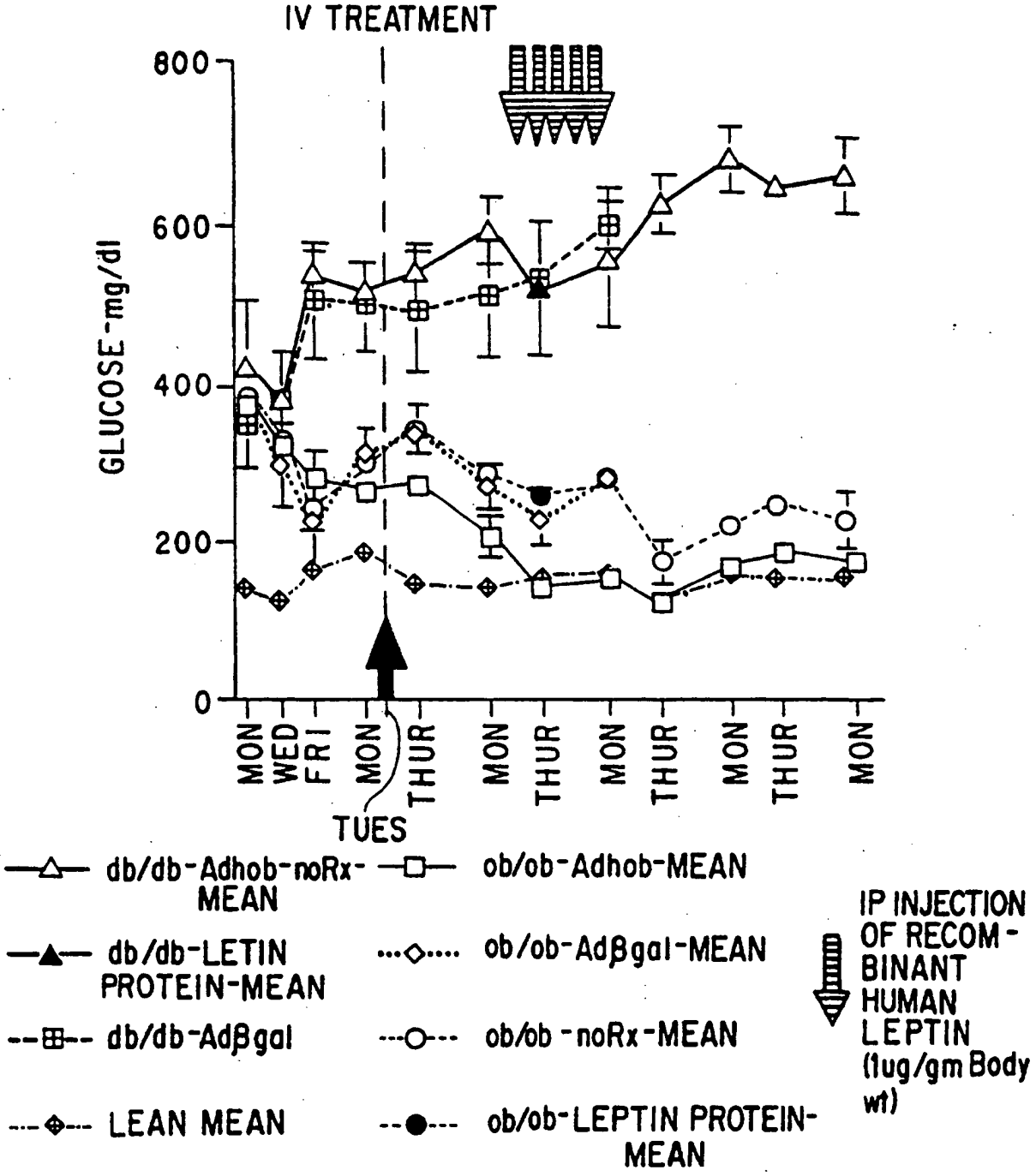

IV TREATMENT
800
600
400
200
0
GLUCOSE-mg/dl
MON
WED
FRI
MON
TUES
THUR
MON
THUR
MON
THUR
MON
THUR
MON
db/db-Adhob-noRx-MEAN
db/db-LETIN PROTEIN-MEAN
db/db-Adβgal
LEAN MEAN
ob/ob-Adhob-MEAN
ob/ob-Adβgal-MEAN
ob/ob-noRx-MEAN
ob/ob-LEPTIN PROTEIN-MEAN
IP INJECTION OF RECOM-BINANT HUMAN LEPTIN (1ug/gm Body wt)

FIG.16

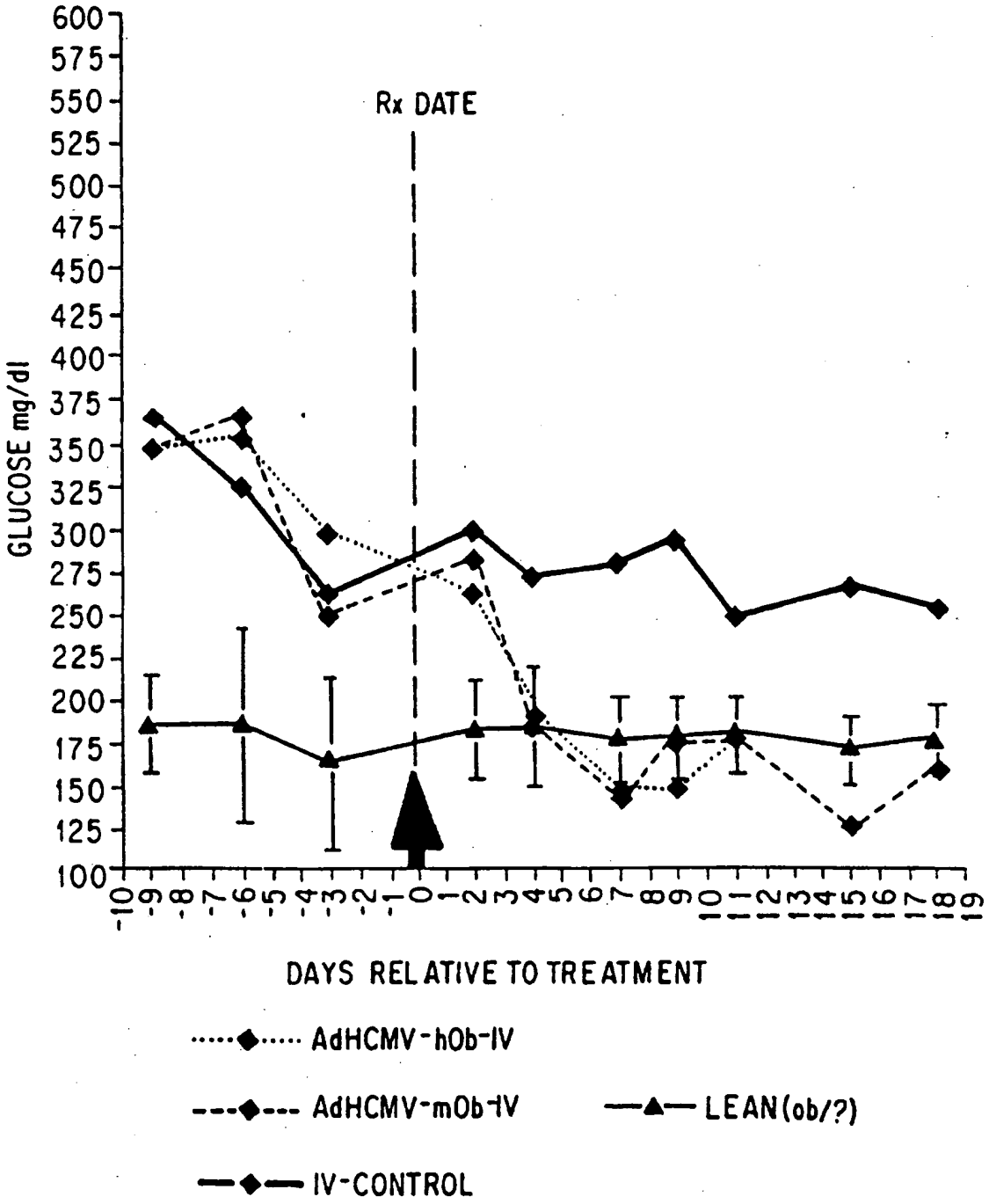

Rx DATE
GLUCOSE mg/dl
600
575
550
525
500
475
450
425
400
375
350
325
300
275
250
225
200
175
150
125
100
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
DAYS RELATIVE TO TREATMENT
AdHCMV-hOb-IV
AdHCMV-mOb-IV
LEAN(ob/?)
IV-CONTROL

FIG.17A

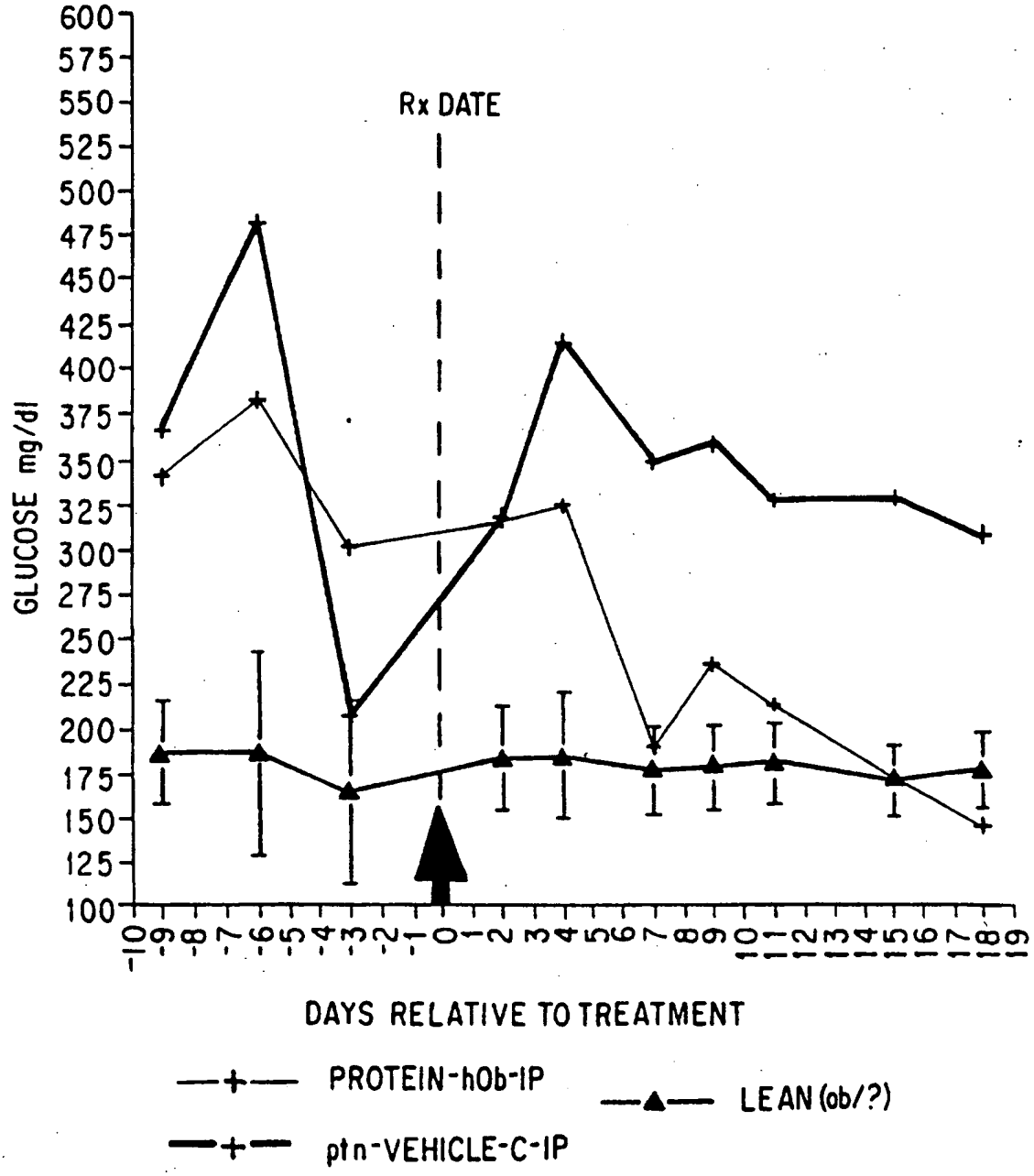
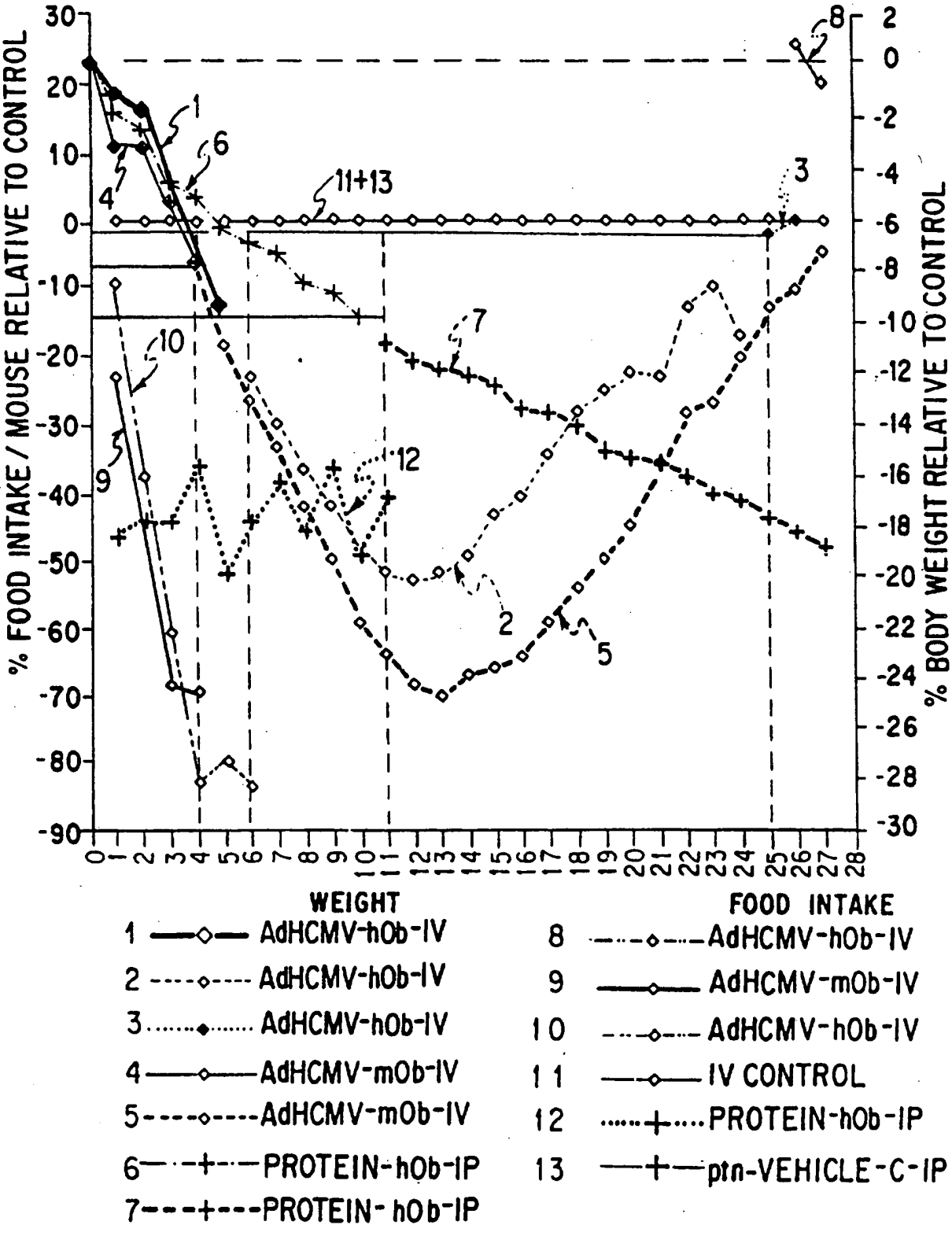

600
575
550
525
500
475
450
425
400
375
350
325
300
275
250
225
200
175
150
125
100
GLUCOSE mg/dl
Rx DATE
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
DAYS RELATIVE TO TREATMENT
PROTEIN-hOb-IP
LEAN (ob/?)
ptn-VEHICLE-C-IP

FIG.17B


% FOOD INTAKE / MOUSE RELATIVE TO CONTROL
% BODY WEIGHT RELATIVE TO CONTROL
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
2
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
-22
-24
-26
-28
-30
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
1
2
3
4
5
6
7
8
9
10
11+13
12
WEIGHT
1 AdHCMV-hOb-IV
2 AdHCMV-hOb-IV
3 AdHCMV-hOb-IV
4 AdHCMV-mOb-IV
5 AdHCMV-mOb-IV
6 PROTEIN-hOb-IP
7 PROTEIN-hOb-IP
FOOD INTAKE
8 AdHCMV-hOb-IV
9 AdHCMV-mOb-IV
10 AdHCMV-hOb-IV
11 IV CONTROL
12 PROTEIN-hOb-IP
13 ptn-VEHICLE-C-IP

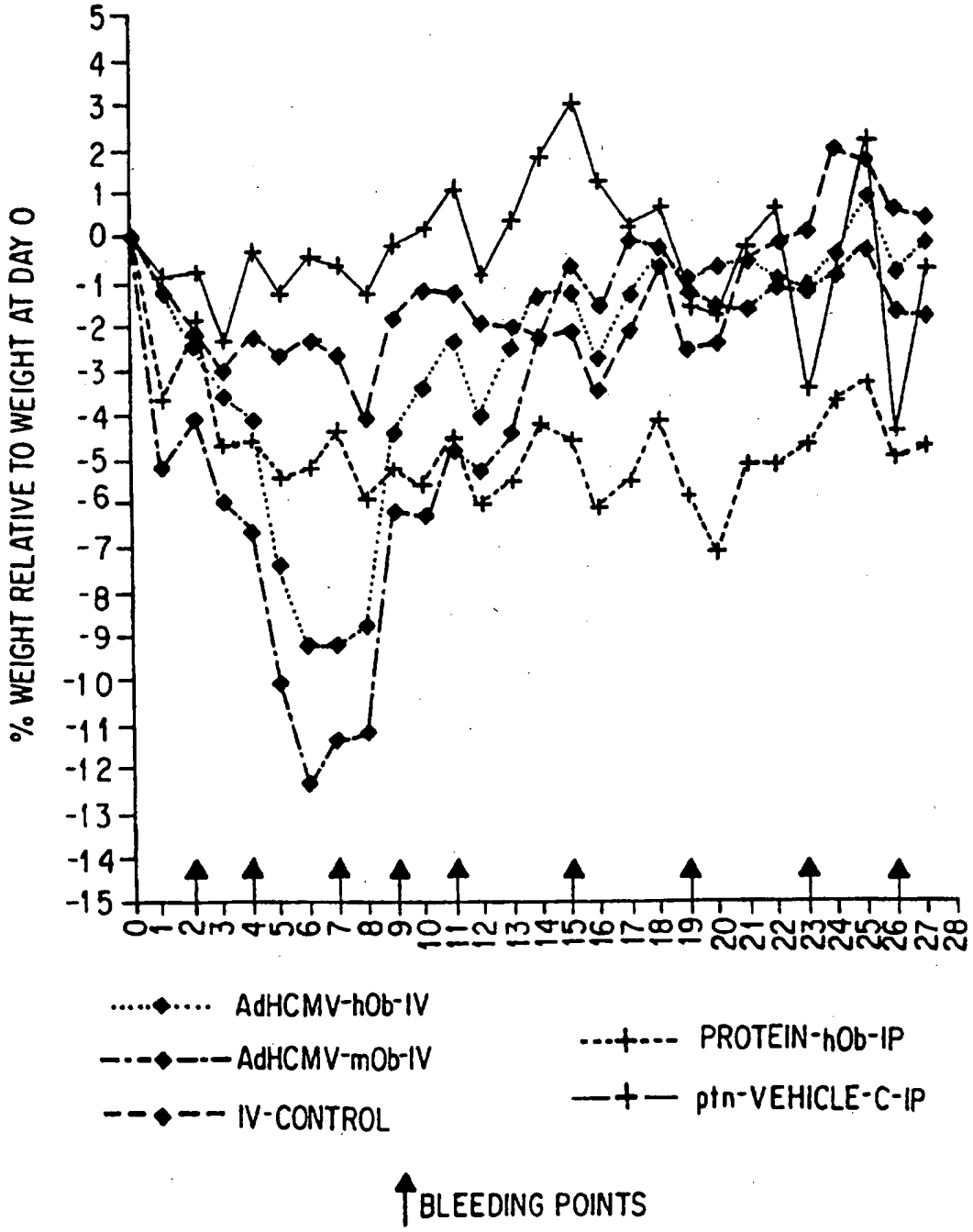

% WEIGHT RELATIVE TO WEIGHT AT DAY 0
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
AdHCMV-hOb-IV
AdHCMV-mOb-IV
IV-CONTROL
PROTEIN-hOb-IP
ptn-VEHICLE-C-IP
BLEEDING POINTS

FIG.19

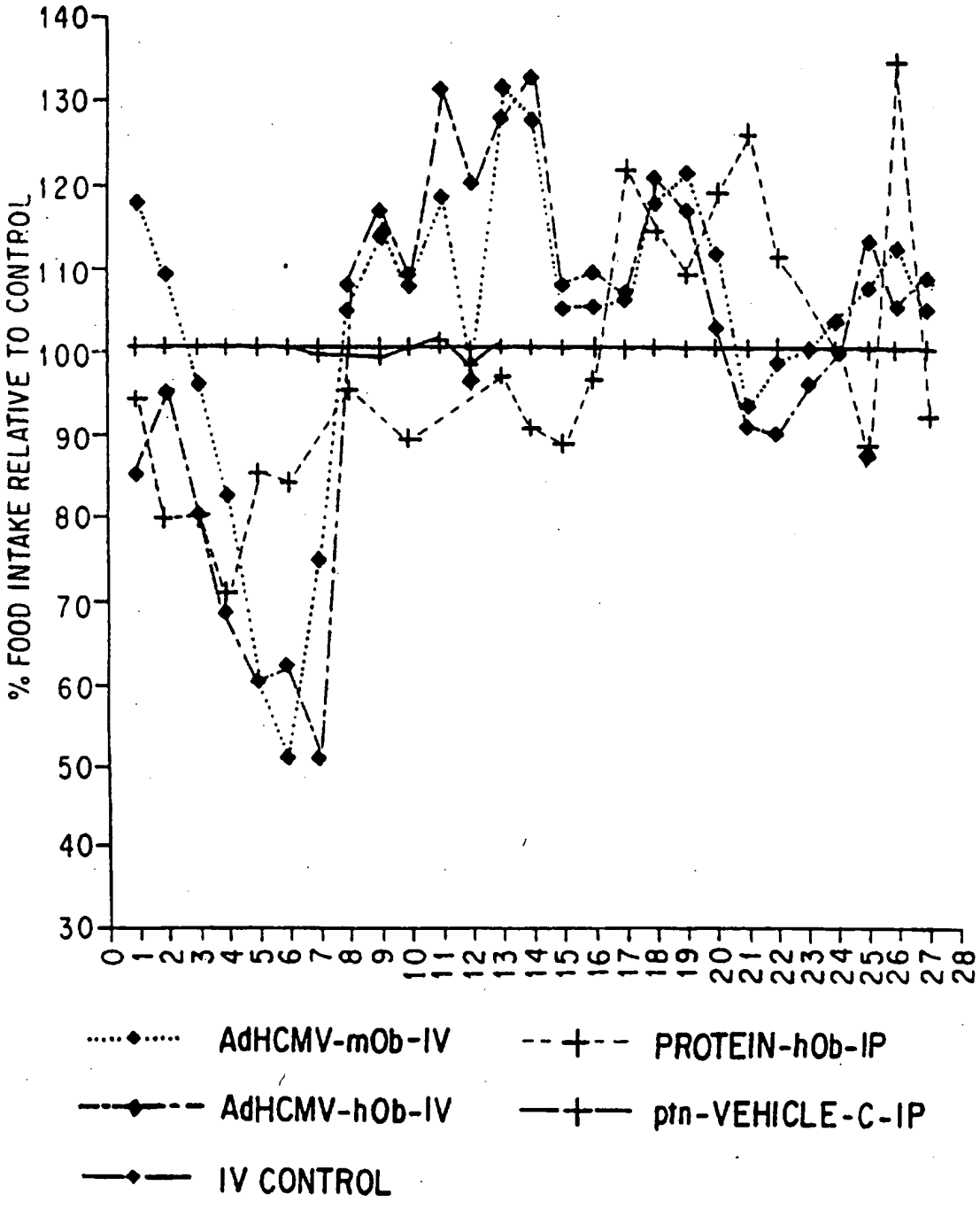

% FOOD INTAKE RELATIVE TO CONTROL
140
130
120
110
100
90
80
70
60
50
40
30
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
AdHCMV-mOb-IV
PROTEIN-hOb-IP
AdHCMV-hOb-IV
ptn-VEHICLE-C-IP
IV CONTROL

FIG.20

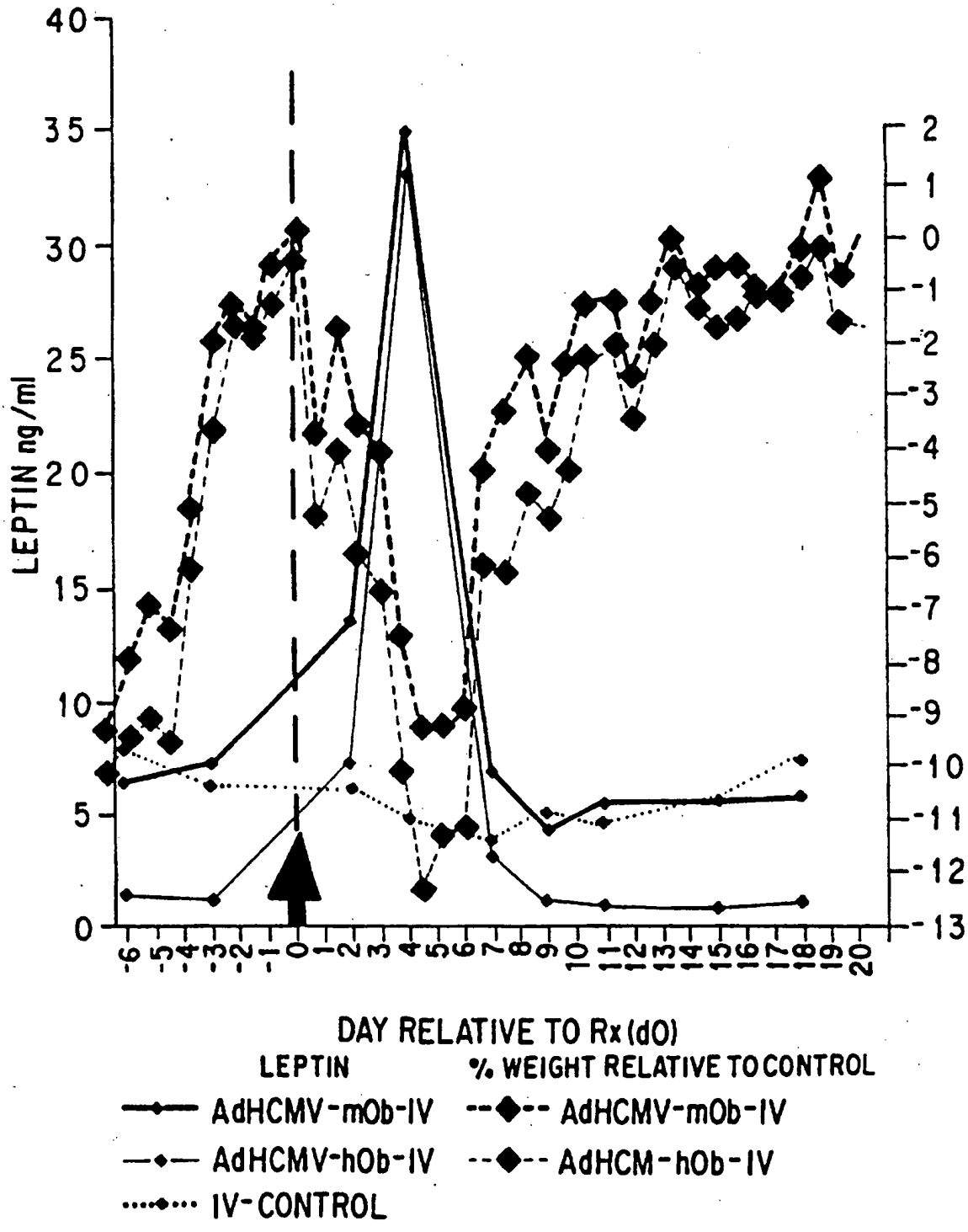

LEPTIN ng/ml
40
35
30
25
20
15
10
5
0
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
-12
-13
-6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
DAY RELATIVE TO Rx (dO)
LEPTIN
% WEIGHT RELATIVE TO CONTROL
AdHCMV-mOb-IV
AdHCMV-mOb-IV
AdHCMV-hOb-IV
AdHCM-hOb-IV
IV-CONTROL

FIG.21A

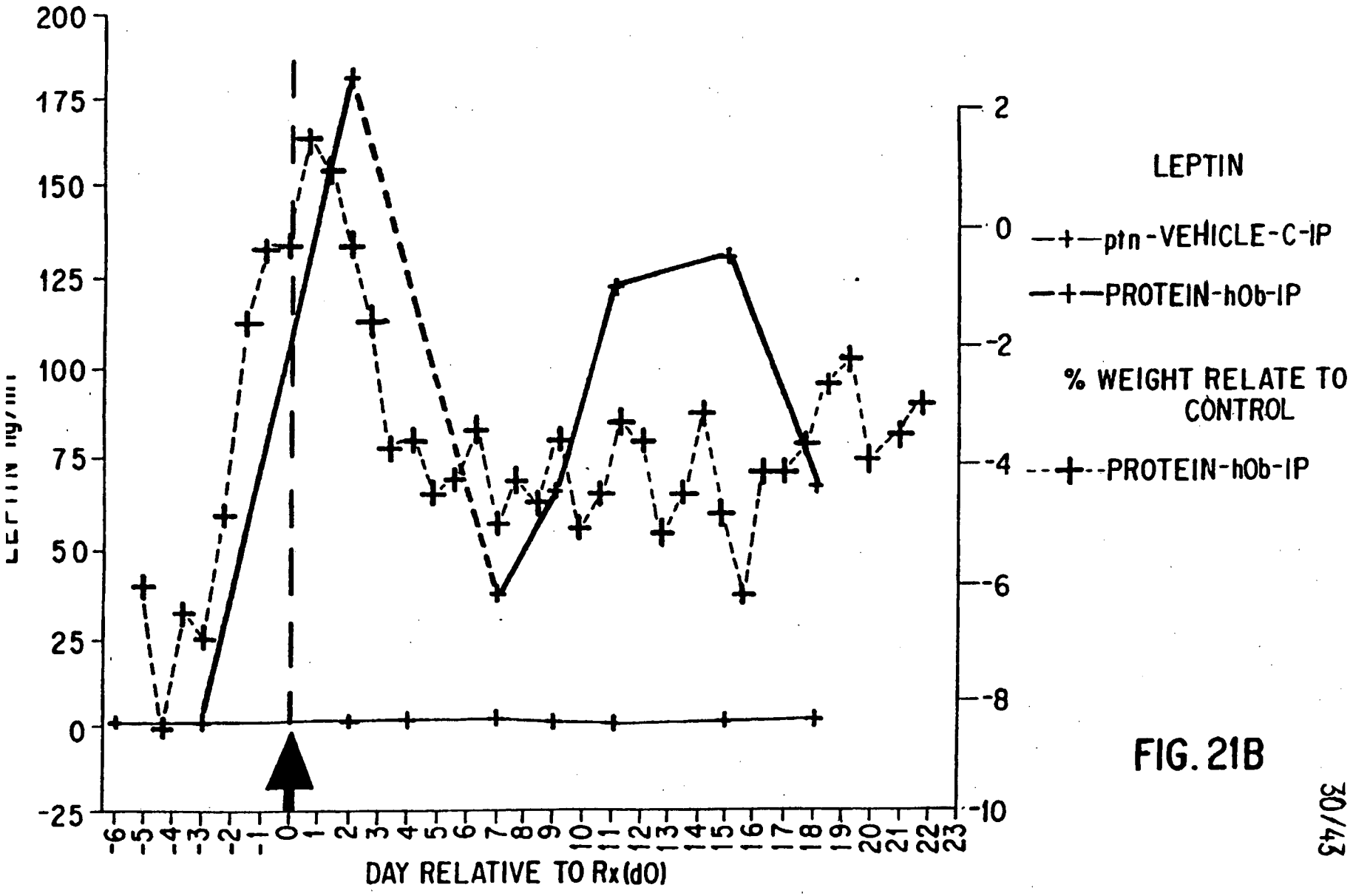

200
175
150
125
100
75
50
25
0
-25
2
0
-2
-4
-6
-8
-10
-6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
DAY RELATIVE TO Rx(d0)
LEPTIN
—+—ptn-VEHICLE-C-IP
—+—PROTEIN-hOb-IP
% WEIGHT RELATE TO CONTROL
--+--PROTEIN-hOb-IP

FIG. 21B

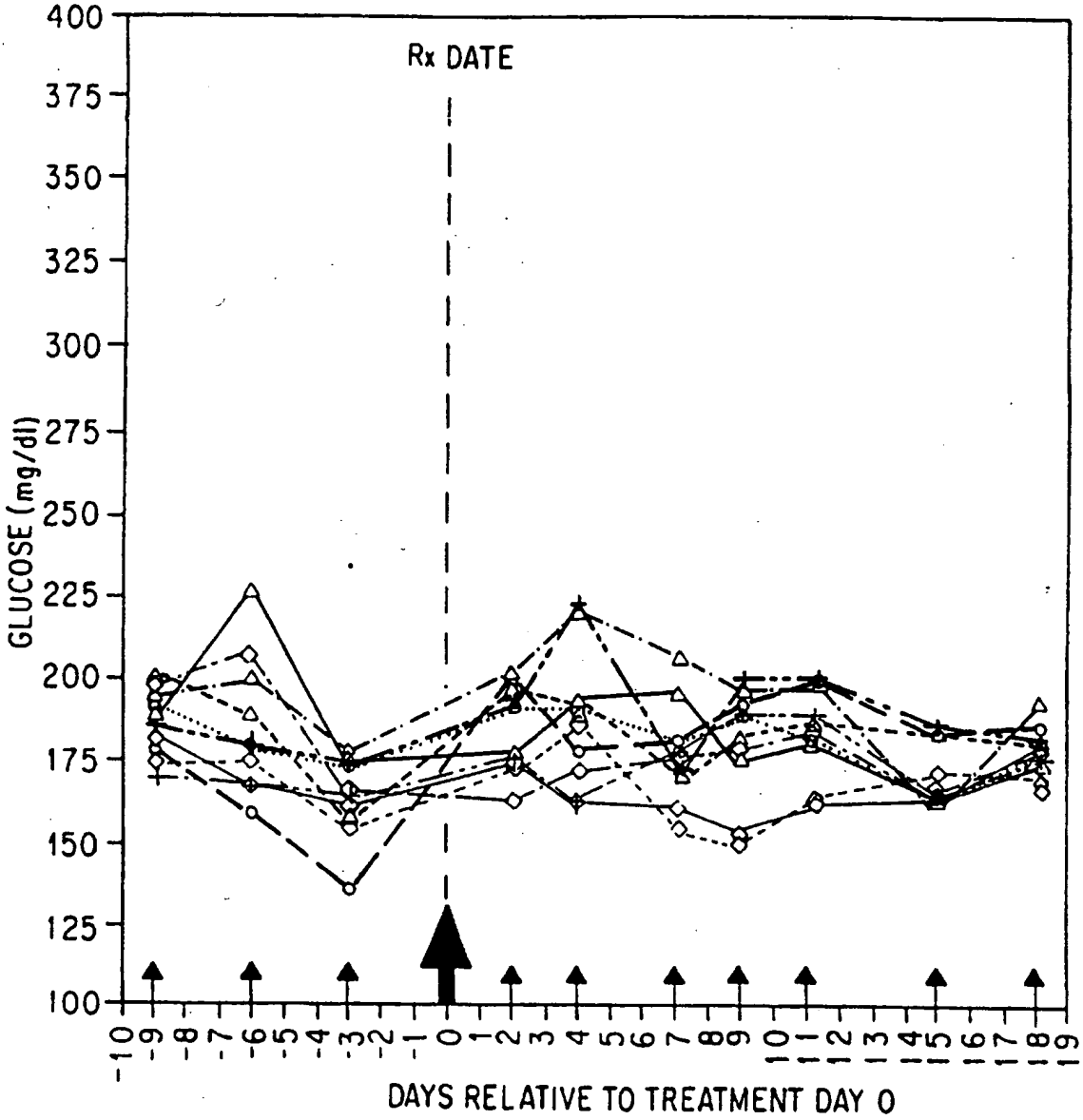

Rx DATE
GLUCOSE (mg/dl)
400
375
350
325
300
275
250
225
200
175
150
125
100
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
DAYS RELATIVE TO TREATMENT DAY 0
AdHCMV-mOb-IV
AdHCMV-β-gal-C-IM
AdHCMV-hOb-IV
pV1Jne-mOb-1M
IV-CONTROL
pV1Jne-CONTROL-1M
AdHCMV-mOb-1M
PROTEIN-hOb-IP
AdHCMV-hOb-1M
ptn-VEHICLE-C-IP

FIG.22

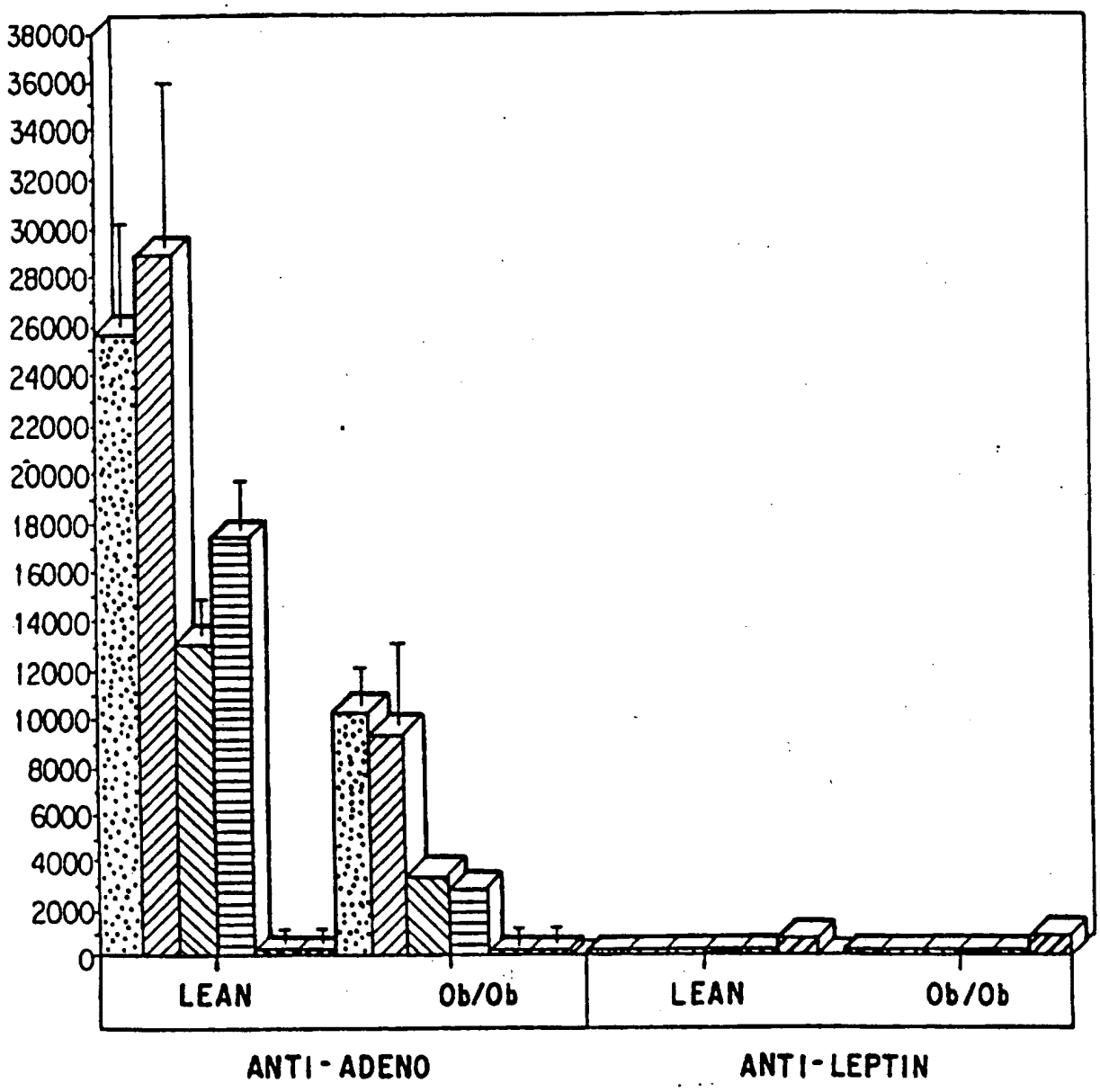

38000
36000
34000
32000
30000
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
LEAN
Ob/Ob
LEAN
Ob/Ob
ANTI-ADENO
ANTI-LEPTIN
AdHCMV-hOb-IV
AdHCMV-mOb-IV
AdHCMV-hOb-IM
AdHCMV-mOb-IM
pV1Jne-mOb-IM
PROTEIN-hOb-IP

FIG.23A

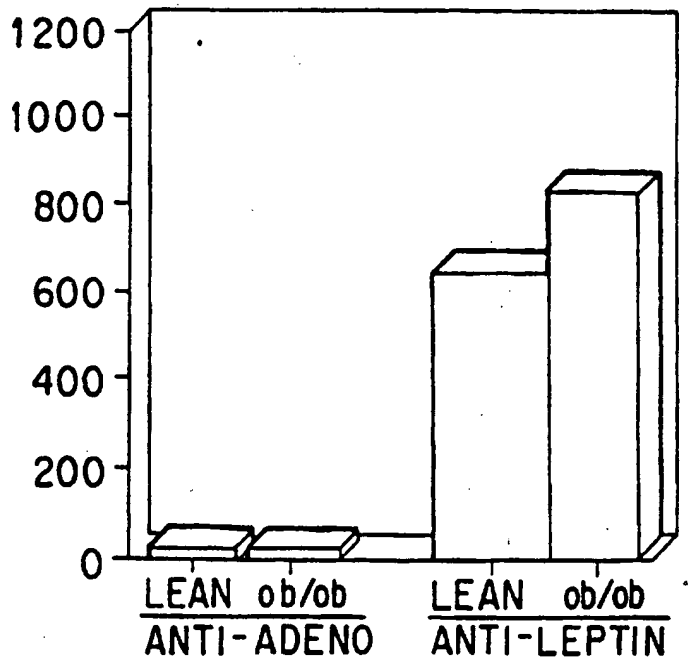

1200
1000
800
600
400
200
0
LEAN ob/ob
ANTI-ADENO
LEAN ob/ob
ANTI-LEPTIN

FIG.23B

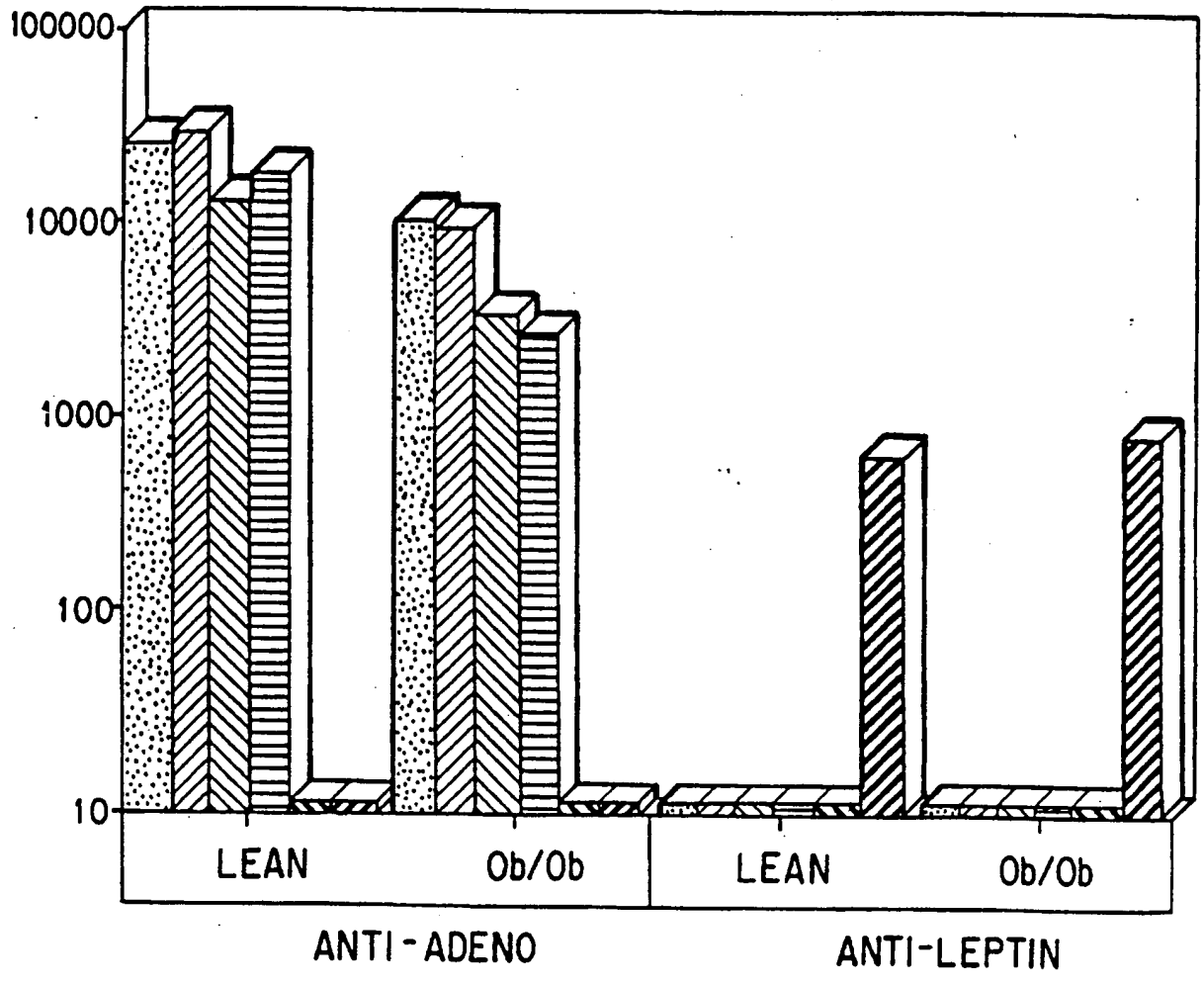

100000
10000
1000
100
10
LEAN
Ob/Ob
LEAN
Ob/Ob
ANTI-ADENO
ANTI-LEPTIN
AdHCMV-hOb-IV
AdHCMV-mOb-IV
AdHCMV-hOb-IM
AdHCMV-mOb-IM
pV1Jne-mOb-IM
PROTEIN-hOb-IP

FIG.24

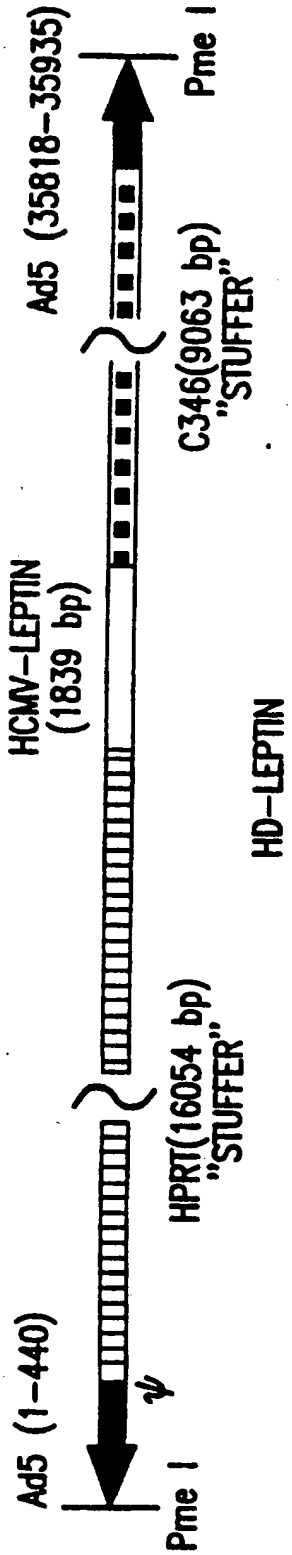


FIG.25A

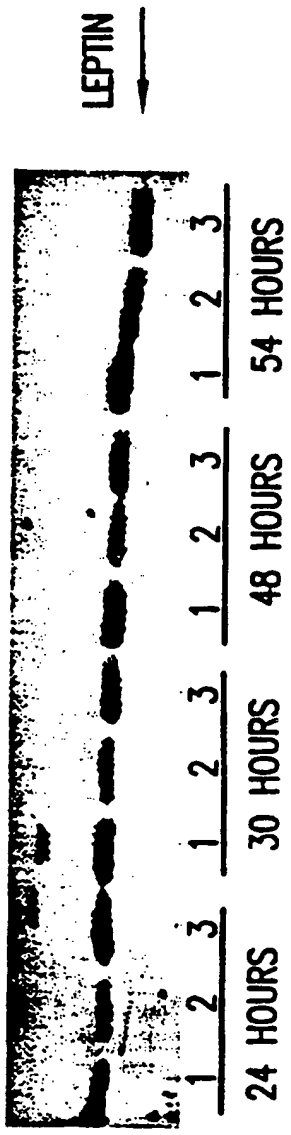


FIG.25B

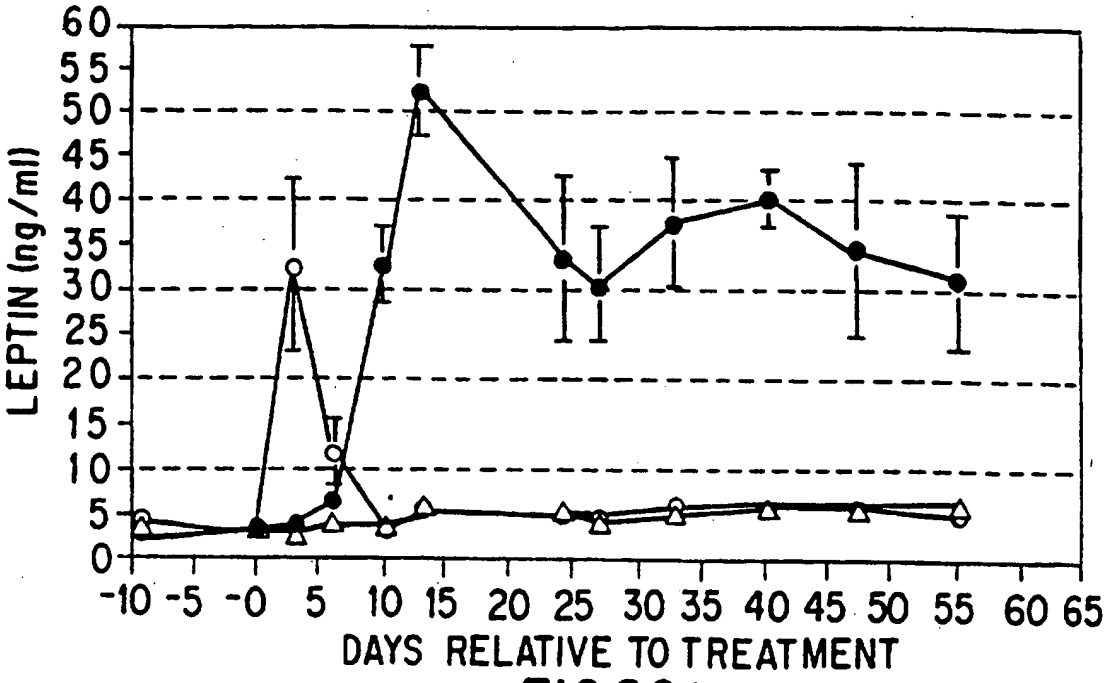


FIG.26A

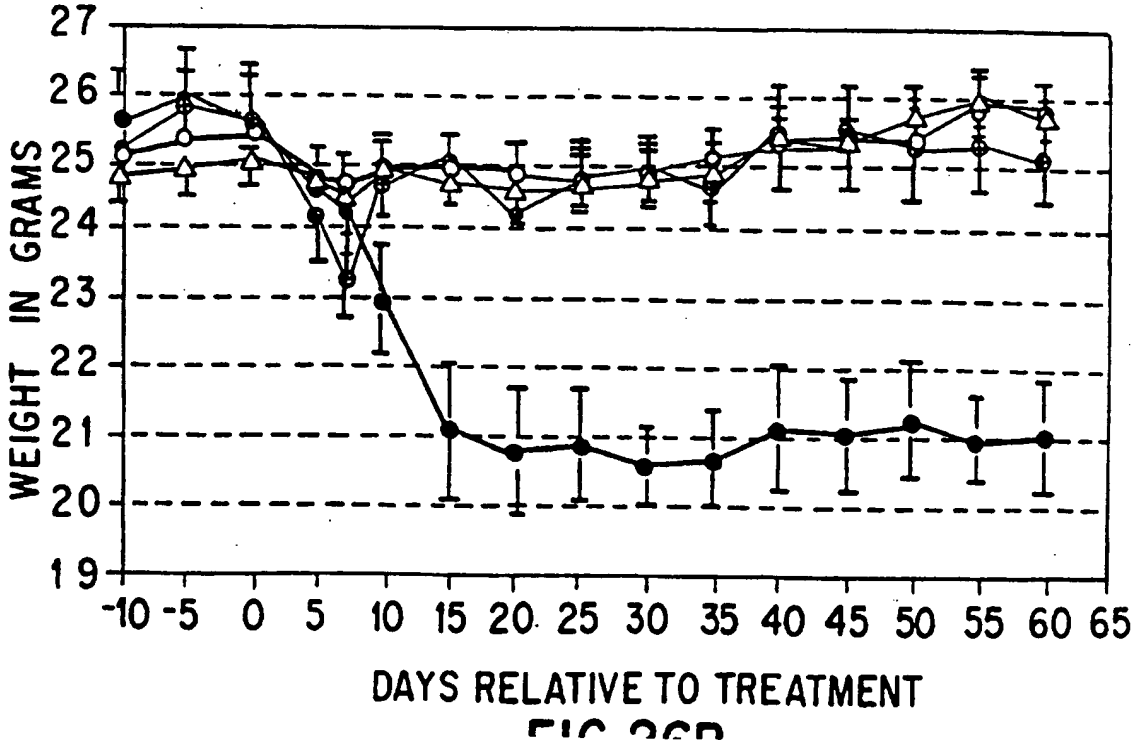


FIG.26B

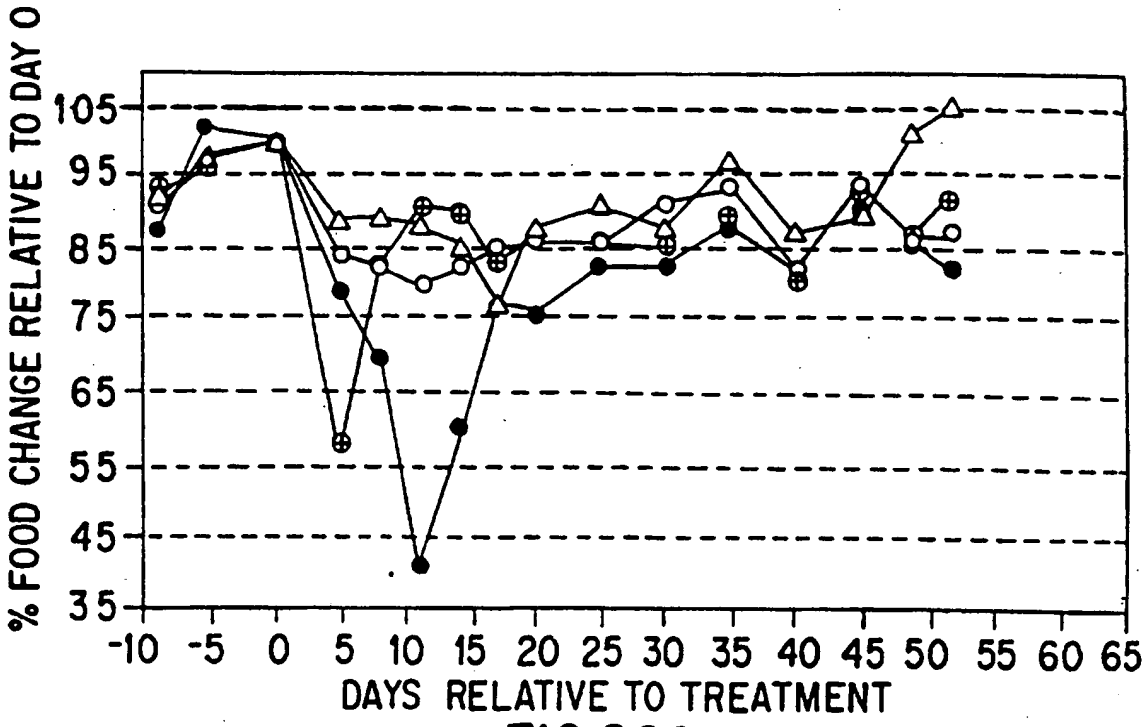
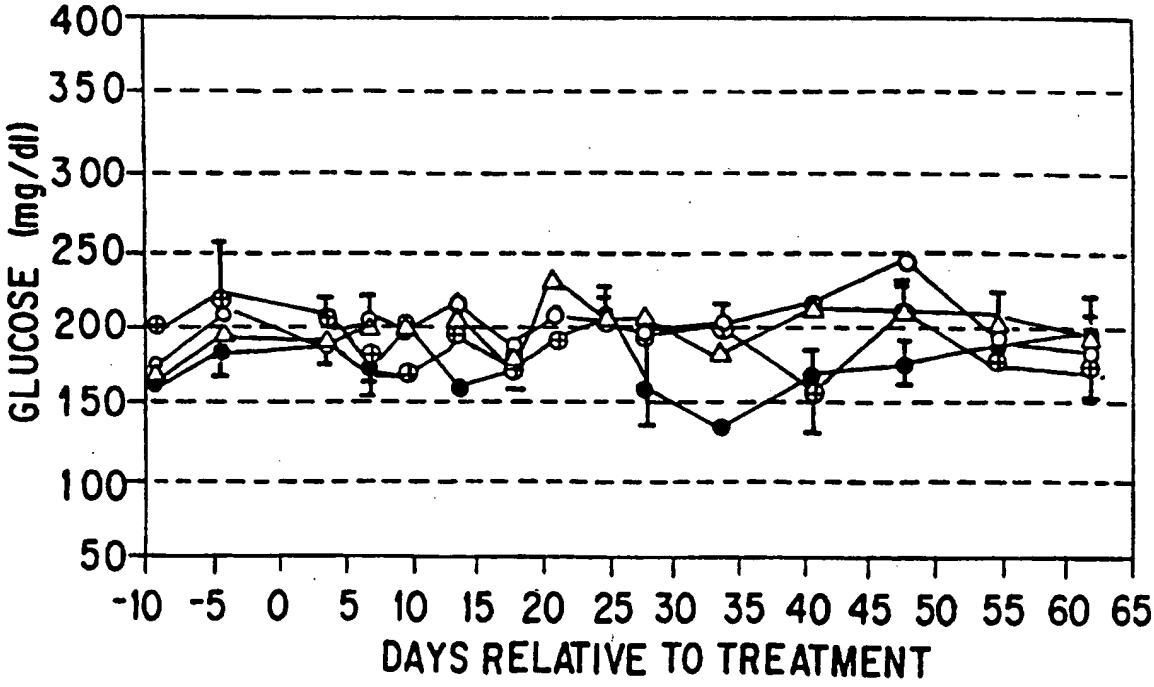

% FOOD CHANGE RELATIVE TO DAY 0
105
95
85
75
65
55
45
35
-10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60 65
DAYS RELATIVE TO TREATMENT

FIG.26C


GLUCOSE (mg/dl)
400
350
300
250
200
150
100
50
-10 -5 0 5 10 15 20 25 30 35 40 45 50 55 60 65
DAYS RELATIVE TO TREATMENT

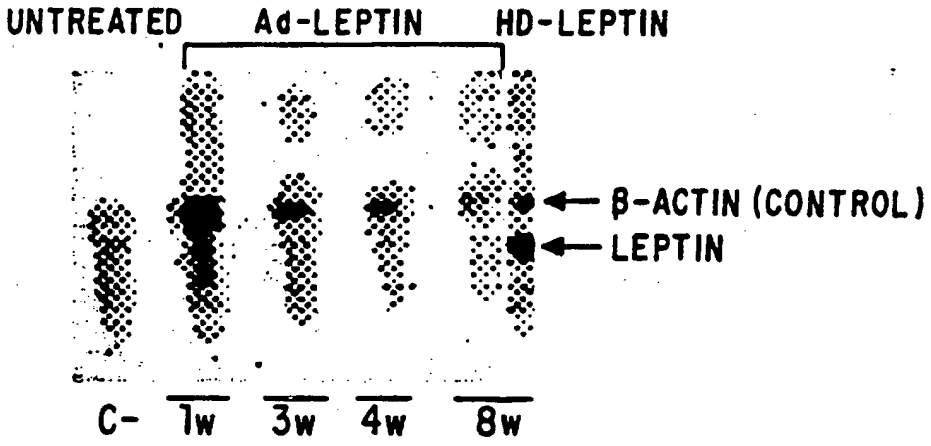

UNTREATED
Ad-LEPTIN
HD-LEPTIN
β-ACTIN (CONTROL)
LEPTIN
C-
1w
3w
4w
8w

FIG.26D

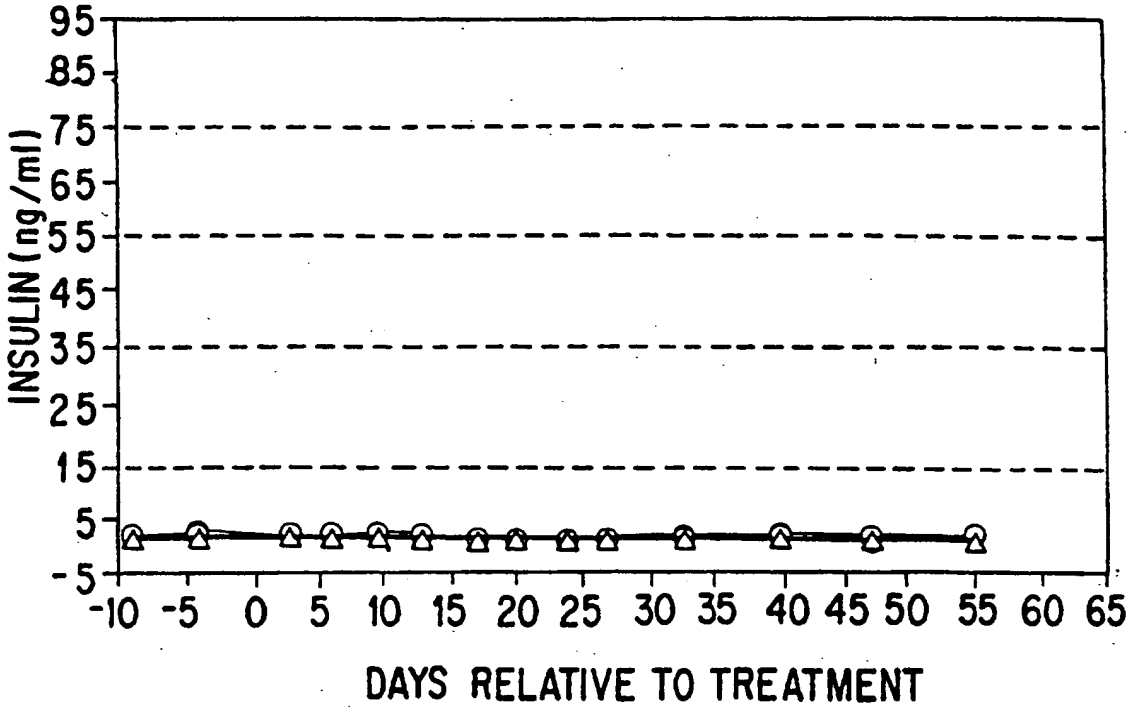


FIG.26F

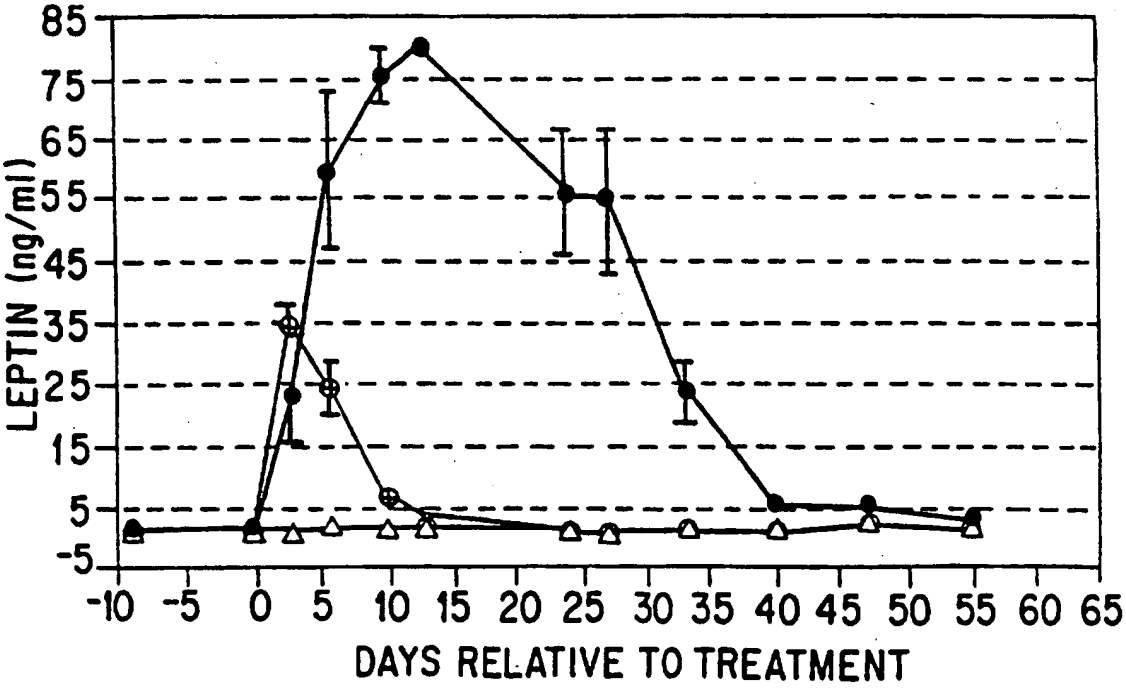


FIG.27A

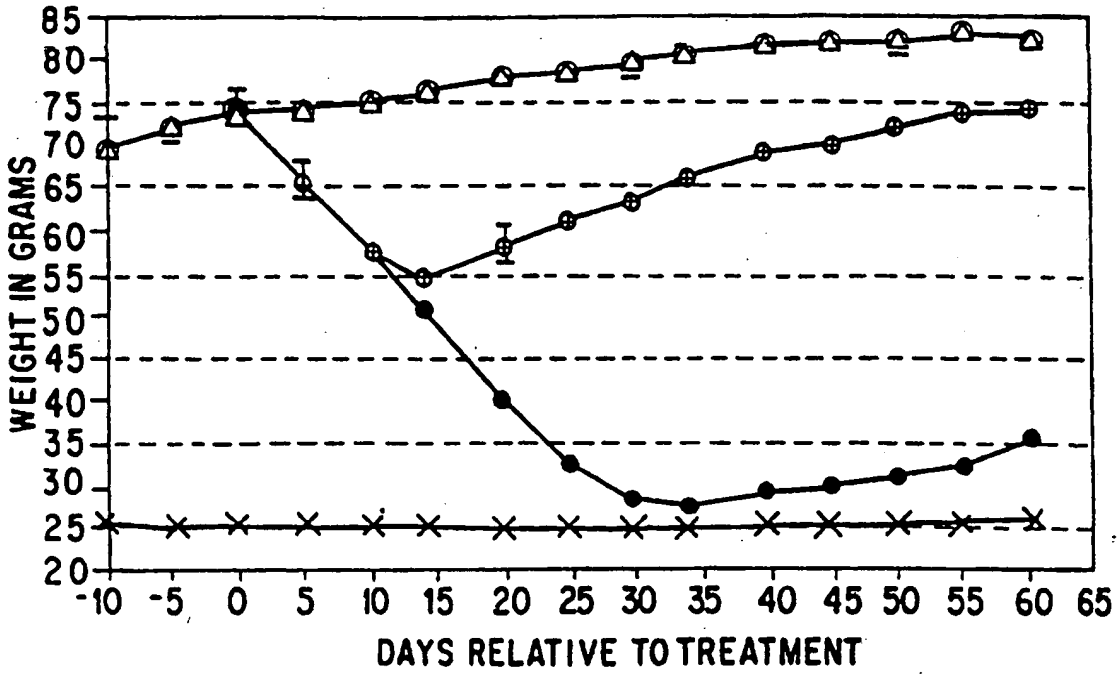


FIG.27B

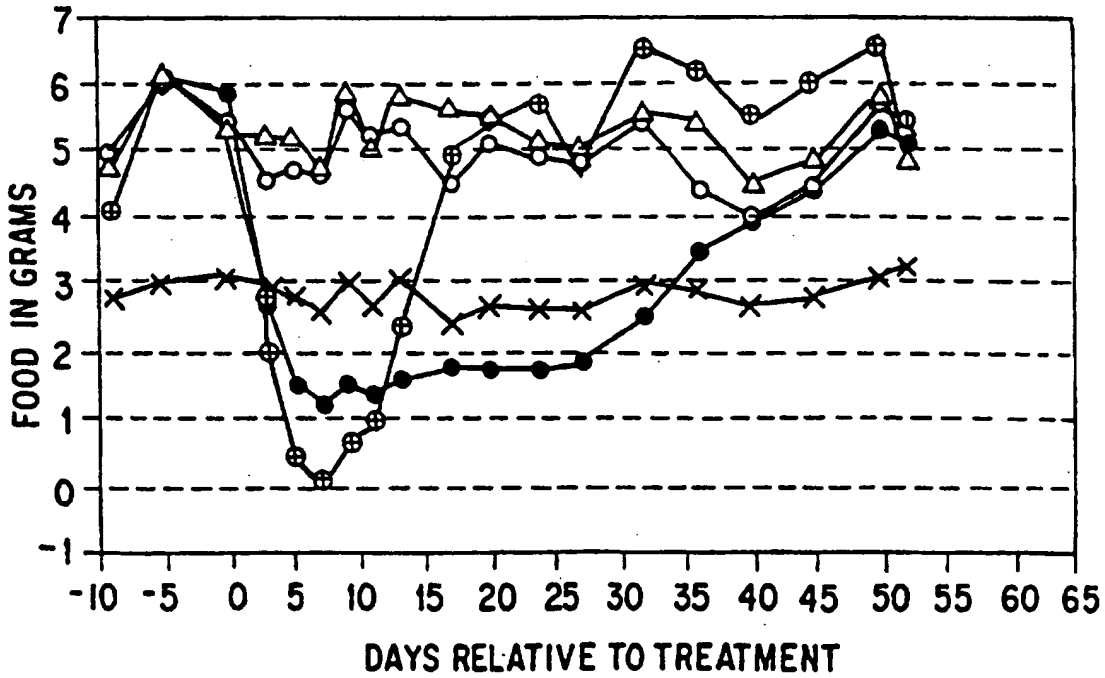


FIG.27C

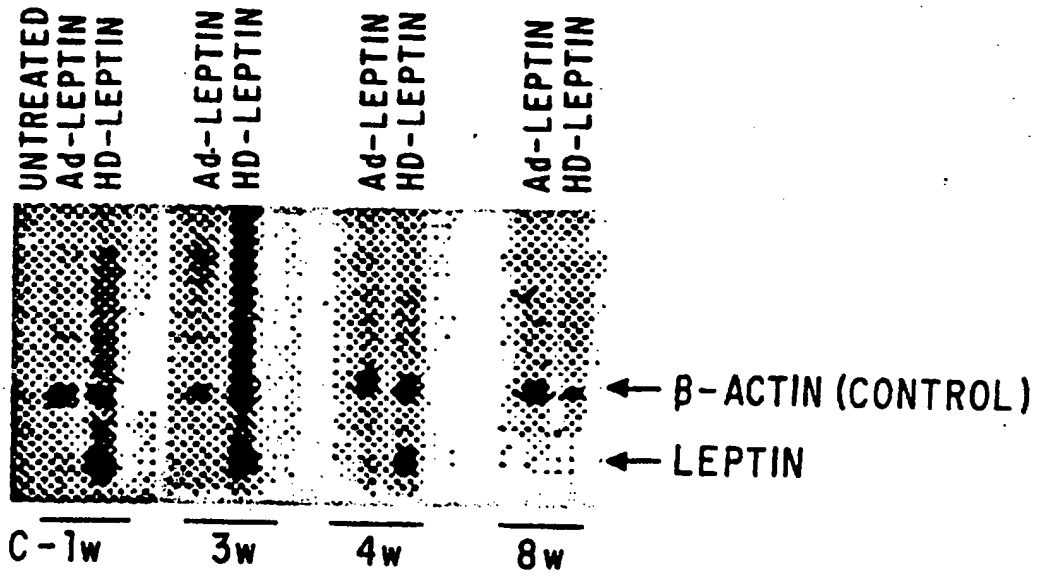

UNTREATED
Ad-LEPTIN
HD-LEPTIN
Ad-LEPTIN
HD-LEPTIN
Ad-LEPTIN
HD-LEPTIN
Ad-LEPTIN
HD-LEPTIN
β-ACTIN (CONTROL)
LEPTIN
C-1w
3w
4w
8w

FIG.27D

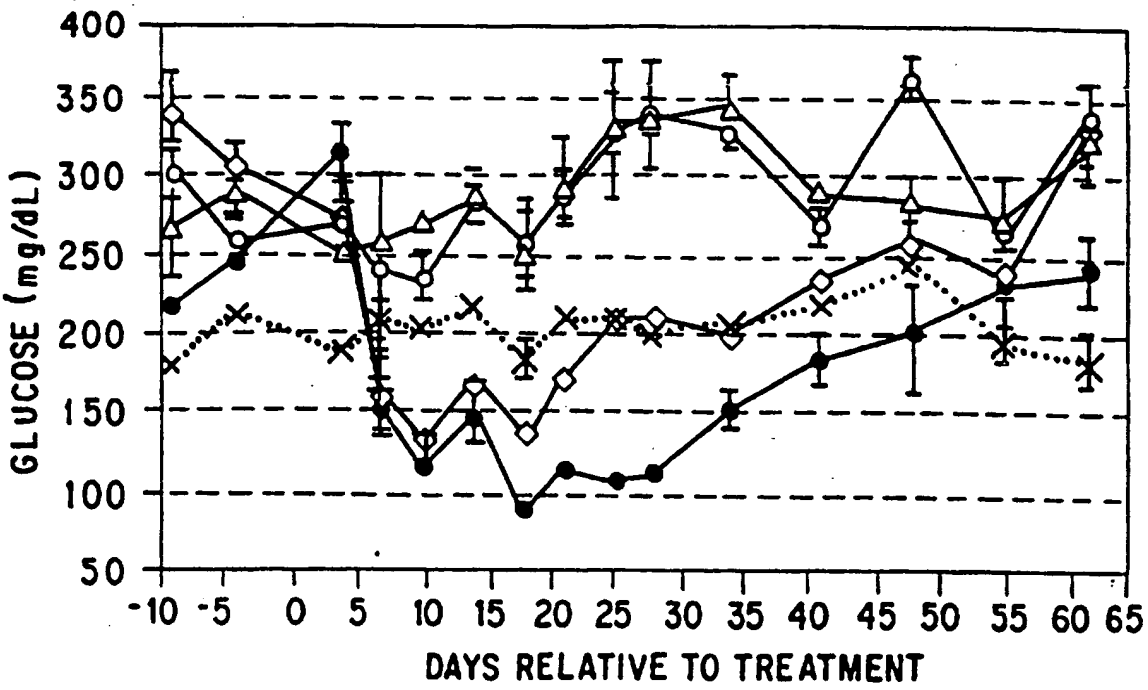


FIG.27E

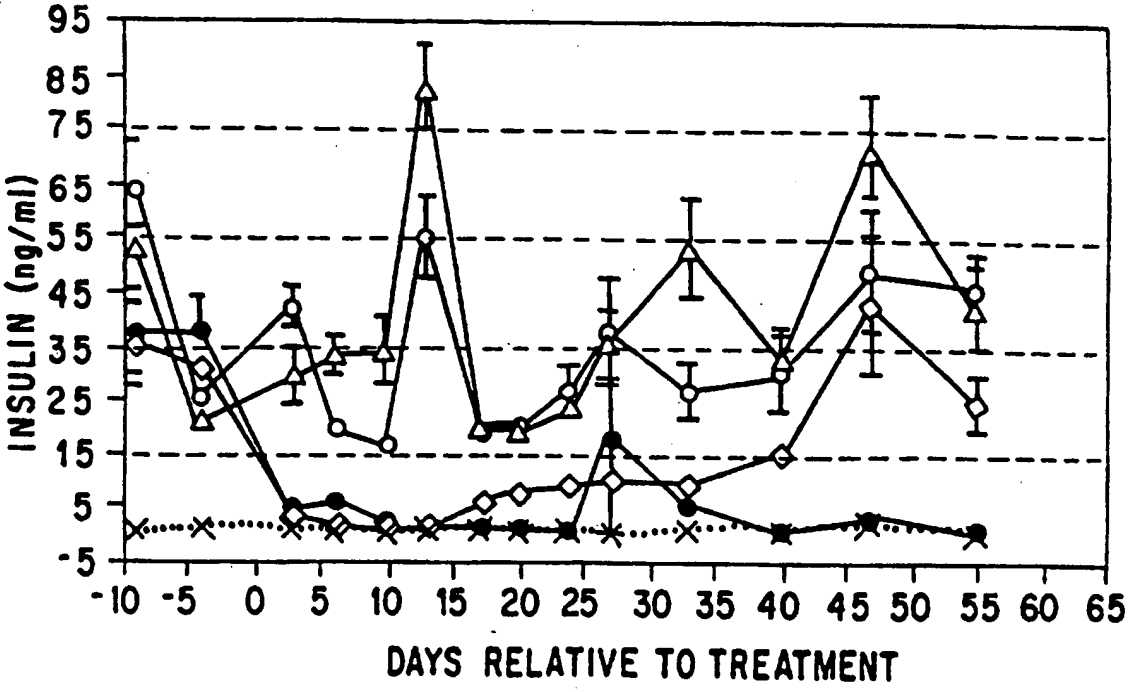


FIG.27F

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/10371

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/16 A61K48/00 A01K67/027 C12N15/86 C12N5/10
G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 292 382 A (UNIV ROCKEFELLER) 21 February 1996 see page 4, line 7 - page 17, line 2 see page 86, line 12 - page 88, line 4 ---	1-16
X	FLETCHER ET AL: "REPLACEMENT GENE THERAPY PHENOTYPICALLY CORRECTS THE FAT DEPOSITION DEFECT IN OB/OB MICE, RESULTING IN NORMALIZED BODY WEIGHT" BLOOD, vol. 86, no. 10S1, 1995, page 241A XP002042614 see abstract 951 --- -/--	1-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 October 1997	22. 10. 97
Name and mailing address of the ISA	Authorized officer

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/10371

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MORGAN R A ET AL: "HUMAN GENE THERAPY" ANNUAL REVIEW OF BIOCHEMISTRY, vol. 62, pages 191-217, XP002031972 see the whole document	1-4
X	WO 92 07943 A (SOMATIX THERAPY CORP ;WHITEHEAD BIOMEDICAL INST (US)) 14 May 1992 see page 5, line 16 - page 7, line 14 see page 8, line 25 - page 14, line 22	1-4
P,X	WO 96 35787 A (CHIRON CORP) 14 November 1996 see page 3, line 18 - page 6, line 24 see page 27, line 3 - page 29, line 8	1-16
P,X	MUZZIN ET AL: "CORRECTION OF OBESITY AND DIABETES IN GENETICALLY OBESE MICE BY LEPTIN GENE THERAPY" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES,USA, vol. 93, December 1996, pages 14804-14808, XP002042615 see the whole document	1-16
P,X	CHEN ET AL: "DISAPPEARANCE OF BODY FAT IN NORMAL RATS INDUCED BY ADENOVIRUS-MEDIATED LEPTIN GENE THERAPY" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES,USA, vol. 93, December 1996, pages 14795-14799, XP002042616 see the whole document	1-16
E	WO 97 32022 A (AMGEN INC) 4 September 1997 see page 3, line 22 - page 8, line 32	1-3,5-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/10371

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2292382 A	21-02-96	AU 3329895 A	07-03-96
		CA 2195955 A	22-02-96
		DE 19531931 A	07-03-96
		EP 0777732 A	11-06-97
		FI 970656 A	17-02-97
		NO 970683 A	16-04-97
		PL 319021 A	21-07-97
		WO 9605309 A	22-02-96
		ZA 9506868 A	09-04-96
		JP 9502729 T	18-03-97
WO 9207943 A	14-05-92	AT 147102 T	15-01-97
		AU 659824 B	01-06-95
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		AU 656544 B	09-02-95
		AU 9017591 A	26-05-92
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		CA 2095256 A	01-05-92
		DE 69123981 D	13-02-97
		DE 69123981 T	05-06-97
		EP 0568537 A	10-11-93
		EP 0556345 A	25-08-93
		ES 2096750 T	16-03-97
		JP 7503121 T	06-04-95
		JP 6503968 T	12-05-94
		WO 9207573 A	14-05-92
WO 9635787 A	14-11-96	AU 5737696 A	29-11-96
WO 9732022 A	04-09-97	NONE	